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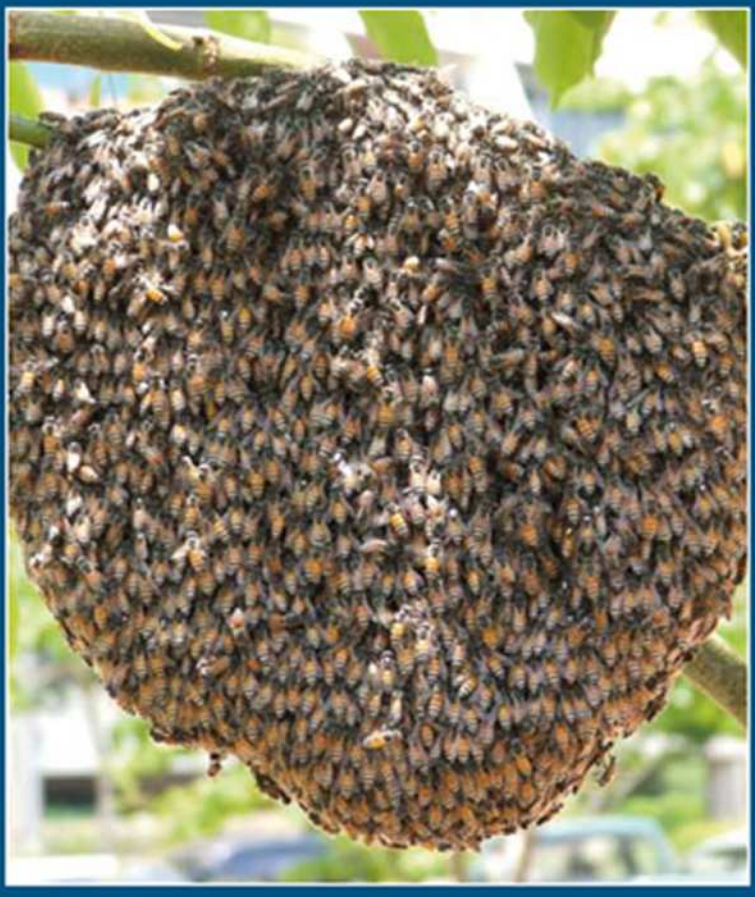

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# Advances in Insect Physiology

*edited by*

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# Behavioural Genetics of the Honey Bee *Apis mellifera*

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## Abstract

The honey bee has long been regarded as an model organism for behavioural genetic studies into the evolution and expression of social traits. The advent of a complete genomic sequence for this insect will now accelerate the discovery of genes that co-evolved with honey bee sociality, and that currently act to regulate the expression of honey bee social behaviour. We review recent progress in the field of honey bee behavioural genetics. First, we discuss various strategies applicable to the honey bee for finding genes associated with variation in social traits. Second, we review the evidence for

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gene-mediated task specialization in individual workers, and show how selection on these genes can affect phenotypes at various levels of biological organization. We make a case study of genes influencing foraging specialization in workers, and identify a variety of candidate genes that are thought to influence the stereotypical maturation from nurse to forager, or influence the tendency for foragers to specialize on nectar or pollen collection. We also discuss the role that genes play in influencing many other behavioural traits, social or otherwise. Finally, we take a look to the future and predict the discovery of genes that underlie truly social traits - namely, those involving altruism. Altruistic traits, like worker sterility, must have evolved through indirect selection acting on non-descendent kin, and the discovery of genes involved in the evolution and expression of these traits will be of great interest owing to the large body of theory that has accumulated regarding their existence. Finally, the honey bee genome opens new opportunities for the genetic improvement of honey bees for commercial purposes via marker assisted selection. We make suggestions for the design of bee breeding programs given the anticipated development of genetic markers for traits of economic interest like hygienic behaviour and defensiveness.

## 1 Introduction

The honey bee, *Apis mellifera*, has long been an organism of choice for studies on behaviour and behavioural genetics. Honey bees have been central to some of the most influential studies in ethology and sociobiology (e.g. Rothenbuhler, 1964; von Frisch, 1967; Lindauer, 1971; Seeley, 1995), while at the same time being an important experimental organism for studies on animal breeding and genetics (Rinderer, 1986; Page *et al*., 2002). Honey bees adapt readily to life in observation hives, can be trained to do certain tasks, and can be manipulated in various ways to see how they react, both at the individual and society level. Honey bees are thus amenable to behavioural genetic studies on cognition, learning and memory, and behavioural thresholds, as well as higher-order social interactions, such as task allocation.

As it is uncomplicated to rear the queen and drone castes, and to control their matings via artificial insemination (Harbo, 1986), it is straightforward to generate large numbers of backcross workers, or produce entire colonies that are genetically homogenous or heterogeneous. Furthermore, the relative ease of production of backcross workers, in combination with an unusually high recombination rate (Hunt and Page, 1995; Solignac *et al.*, 2004), permits very fine-scale genetic mapping (e.g. Hasselmann *et al.*, 2001), which allows for genes affecting variation in behavioural traits to be localized to relatively narrow genetic regions. Thus, honey bees are very useful for studies in behavioural genetics.

More recently, a suite of genomic tools has been developed for the honey bee, including nearly saturated genetic maps based on RAPDs (Hunt and Page, 1995), microsatellites (Solignac *et al.*, 2004), and AFLPs (Rueppell *et al.*, 2004a), and a now-complete genomic sequence (Honey Bee Genome Sequencing Consortium, 2006). Add to this several independently constructed gridded genomic (Beye *et al.*, 1998) and expression tag (Whitfield *et al.*, 2002) arrays, as well as a high-density oligonucleotide microarray (Honey Bee Genome Sequencing Consortium, 2006), and it is clear that new opportunities for detailed studies on honey bee behavioural genetics are upon us, as well as opportunities for far-reaching comparative studies against other model taxa, such as *Drosophila* and humans.

Despite their many advantages as a model organism for behavioural genetics, it must be acknowledged that honey bees also come with several disadvantages, especially when compared with *Drosophila*. Chief among these is the relatively lengthy generation time: at least eight weeks from colony to daughter colony. This hinders our ability to inbreed or select recurrently. A second disadvantage is the very severe effects of inbreeding caused by the genetic load imposed by the complementary sex determining locus (Beye *et al.*, 2003). The negative effects caused by inbreeding mean that maintenance of mutants or selected lines is tedious, expensive and usually unsuccessful in the long term. Furthermore, honey bees are difficult to propagate in enclosures and impossible in the laboratory setting. This means that the maintenance of transgenic lines is unlikely to meet with regulatory approval, as containment is incompatible with long-term viability. Finally, honey bee colonies are physically large and expensive to maintain, their propagation is seasonal, and because the workers sting, a large number of researchers and technicians prefer to avoid working with them.

As the era of genomics unfolds, this new review of honey bee behavioual genetics is timely. In it we have attempted to promote the honey bee's extended role as a model organism in behavioural genetic research and outline general strategies that are being used to locate genes related to behaviour and, particularly, social behaviour. Furthermore, as honey bees are the basis of major honey production and pollination industries, we have also attempted to outline how the new tools of genomics could be applied to assist practical bee breeding.

## 2 Colony versus individual phenotypes

In discussing honey bee behavioural phenotypes, it is useful to distinguish between different levels of biological organization (Fig. 1). We follow Reeve and Keller (1999) and regard the gene as the fundamental unit of selection (*replicator*) and the individual, patriline, and colony as potential packages (*vehicles*) in which genes are expressed, and therefore on which selection

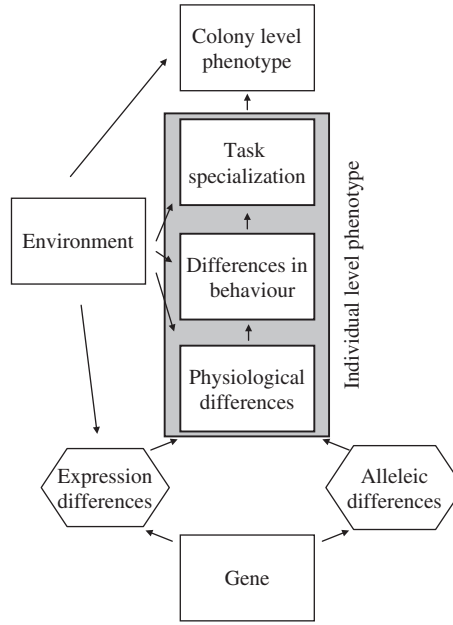


FIG. 1 Levels of biological organization between the gene, individual, and colony or society. Arrows roughly reflect the direction of cause and effect. From a gene-centric perspective, genes can vary in their sequence or expression pattern, and these differences can affect the physiology of individuals, resulting in differences in behaviour. Variations in an individual's behaviour, such as that affecting task specialization, can in turn lead to variations in colony-level phenotypes.

directly acts. Social phenotypes provide a model to study how selection may act simultaneously at different levels – a phenomenon that is perhaps best understood in honey bees (Seeley, 1997). For example, the amount of pollen a colony collects and stores in its brood nest is clearly a colony-level behavioural phenotype that is readily quantified. Beekeepers can recognize high pollen-hoarding colonies, and high and low pollen-hoarding strains can thus be selected by recurrent selection on the colony-level phenotype (Hellmich *et al.*, 1985; Page and Fondrk, 1995). However, if we dig a little deeper, we find that the average worker from a high pollen-hoarding strain shows a set of behavioural and physiological characteristics that is different from that found in an average worker from low pollen-hoarding strain. For example, selection at the colony-level phenotype of pollen hording changes a host of worker-level characters including: response to sucrose concentration (Pankiw and Page, 1999; Pankiw *et al.*, 2002), the size of typical nectar and pollen loads collected (Pankiw *et al.*, 2002), age at the onset of foraging (Calderone and Page, 1988, 1996), preferences for nectar and pollen foraging (Page *et al.*, 1995; Fewell and Page, 2000), levels of circulating juvenile

hormone in young bees (Pankiw and Page, 1999; Schulz *et al*., 2004) and differing levels of neurochemicals in the brain (Humphries *et al*., 2003).

A second example of this phenomenon is selection for hygienic colonies that rapidly remove dead brood. Such colony-level selection results in changed worker characteristics, particularly the ability of workers to learn and respond to the odour of dead brood (Masterman *et al*., 2000, 2001). Conversely, selection on individual worker phenotype can also change a colony-level phenotype. For example, selection for worker reproduction by breeding from worker-laid males results in dysfunctional colonies in which there is breakdown of social cohesion (Barron *et al*., 2001). These examples illustrate how important it is to not only distinguish between higher-level and individual phenotypes, but also to simultaneously recognize how subtle differences in behaviour at one level can result in significant shifts in behaviour at another level.

## 3 Finding genes for behavioural traits

The goal of behavioural genetics is transforming; from simply demonstrating (statistically) that variance in behaviour has a genetic component to revealing the actual genes that are causally associated with behavioural variation (Robinson *et al*., 1997), and even describing the dynamic interplay that is expected to occur between genotype, phenotype and environment (Jackson *et al*., 2002). Genomic tools such as microarrays and complete genomic sequences are, for example, allowing us to begin making links from quantitative to qualitative genetics, and ultimately to dissect gene-behaviour relationships by uncovering the genes and gene products that influence behaviour.

To begin finding genes for behavioural traits, we require a search strategy. The strategy chosen will depend on two key considerations: (1) the amount of information in hand regarding the genetic architecture of the trait, and (2) the availability of genomic resources for the target organism. If little is known about the potential genes involved (this will unfortunately be true in most cases) and there are few genomic resources available, then the power to identify functionally important genes will initially be low and a general search pattern will have to be adopted (Vasemagi and Primmer, 2005). However, as knowledge of the molecular underpinings of behaviour begin to emerge, and as more genomic tools and resources become available, in particular for honey bees, the power to identify genes affecting their behaviour will correspondingly increase.

### 3.1 REVERSE GENETICS

One approach for finding behavioural genes is to first develop a short-list of candidates, usually with reference to their known or presumed function in

another organism. The candidate genes are then tested, to the exclusion of other genes, for involvement in the behaviour. Though selective in terms of loci screened, this 'reverse genetic' strategy is expedient, and makes use of the fact that a gene's molecular function tends to be conserved across taxa. Thus, information regarding gene function in one taxon (e.g. *Drosophila*) can be exported to another taxon (e.g. *Apis*), at least provisionally.

One example of a targeted screen involved testing *malvolio* (*mvl*) as a contributing factor affecting foraging behaviour in honey bee workers (Ben-Shahar *et al*., 2004). In this study, *mvl* was chosen as a candidate because, as a manganese transporter, it is known to influence responsiveness to sucrose in *Drosophila*, and variation in sucrose response among individuals is known to influence foraging-related task-specialization in honey bees (see Section 4.3.2). Ben-Shahar and colleagues showed that the levels of *mvl* mRNA in the brain cells of workers are strongly associated with differences in worker foraging activity: pollen foragers tend to have higher levels of *mvl* transcript than nectar foragers and foragers of either type have higher levels than do non-foraging nurse bees. It appears that some feeding-related genes in *Drosophila* are also related to feeding in *Apis*, and in the case of honey bees may be related to age-based task specialization.

The association between gene expression and behaviour is sufficiently strong for these authors to suggest that *mvl* is a component in a molecular pathway linking a worker's perception of food quality to division of labour within the colony. This is an example of a gene conserved in function from a distantly related (approximately 300 MY) non-social taxon affecting, or is at least correlated with, variation in honey bee behaviour. In the *Drosophila–Apis* case, the *mvl* and also *for* gene (Ben-Shahar *et al*., 2002) appear to have something to do with the regulation of foraging behaviour, but their precise role or importance is not yet known with certainty (Rueppell *et al*., 2004a). The important task of expanding this type of single-locus genetic information to a complete molecular pathway will be the next step. However, we mentioned this example here because it shows that, in general, the candidate gene approach (Fitzpatrick *et al*., 2005) has promise in behavioural genetics – despite the fact that it is restricted only to those genes that are conserved from other model taxa.

## 3.2 FORWARD GENETICS

What if taxon-specific genes are sought, or if no candidate genes are otherwise available? In these cases, it is still possible to make inroads – by screening as many loci as possible. In contrast to the candidate gene approach, where specific genes are hypothesized in advance, open-ended screens will ideally cover the whole genome. This maximizes the chance of detecting differences in genotype or gene expression that are associated

with differences in behaviour, and without making specific predictions about which genes are potentially important. The absence of candidate genes in a forward type screen is both an asset and a liability. Open-ended screens do potentially reveal novel genes. On the other hand, forward approaches tend to generate a large number of candidates that must each in turn be individually evaluated for their potential involvement in the behaviour of interest. Moreover, the large number of tests involved when screening large numbers of genes – say, using a microarray – creates statistical challenges in the form of false discoveries, which need to be strongly controlled for (Smyth, 2004), but at the expense of power.

Whitfield *et al.* (2003) used a forward screen to identify a previously unknown set of genes associated with task specialization in honey bee workers. By comparing microarray-generated expression profiles across ~5500 loci, Whitfield *et al.* discovered ~50 genes for which change in transcript abundance was strongly predictive of an individual's behavioural class, regardless of age. In this case, the behaviours were nursing and foraging, which in natural colonies represent end-points of an age-related within-caste polyethism; young workers normally care for the brood and can be considered as nurses, while older workers forage (Winston, 1987). Whitfield *et al.*'s list of genes is full of new candidates for age-based behavioural transitions. Some of these genes have passed initial follow-up tests in that they have subsequently been associated with pheromone-mediated transitions from nurse to forager (Grozinger *et al.*, 2003).

### 3.2.1 *Linkage mapping*

Quantitative analysis of genetic effects on behavioural traits can help reveal the presence and location of genes of major effect, their epistatic interactions, and can potentially verify the effects of already-known candidates. In cases where heritable differences in behavioural phenotypes involve a few loci of major effect, as opposed to many loci of small effect, then an obvious target for behavioural genetic studies are the 'major' genes, those that explain the greatest amount of phenotypic variation.

Quantitative trait loci (QTLs) are genomic regions that contain one or more genes that contribute to phenotypic variation of quantitative traits, as opposed to traits that vary in a qualitative manner. QTLs can be identified by a procedure known as linkage mapping. The principles behind linkage mapping in a backcross are conceptually straightforward. Two lines that differ strongly in a phenotype of interest (in this case, a behavioural phenotype) are crossed to produce an $F_1$. The $F_1$ individual is then backcrossed to produce an array of progeny that segregate into two genotypic classes, homozygous and heterozygous (Fig. 2). The progeny are each scored phenotypically for the trait of interest and then genotyped at a large number of molecular marker loci (e.g. microsatellites or SNPs). If

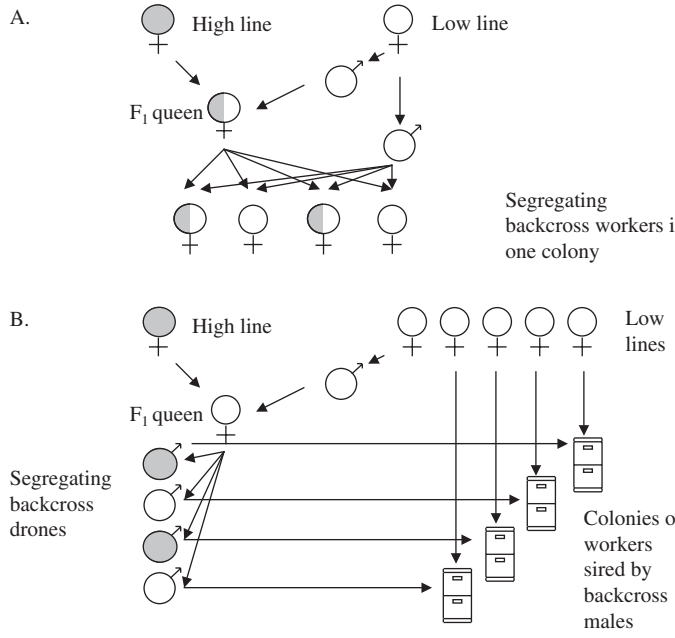


FIG. 2 Simple backcross schemes used to generate segregating populations of workers (A) or colonies (B). (A) Two inbred parental lines of contrasting phenotype, 'high line' (shaded) and 'low line', are crossed to produce a heterozygous F1 queen. She is backcrossed to a (haploid) drone of the presumed recessive parent ('low') to produce a cohort of offspring that segregate within a single colony for the phenotypically variable trait. Workers homozygous for the trait of interest should show the 'low' phenotype, whereas heterozygotes should show the 'high' phenotype. If the trait is controlled by a single locus, the ratio of the two phenotypic classes should be roughly 1:1. (B) As above, except that the heterozygous F1 queen is used to produce unfertilized eggs that develop as haploid drones. These males are then used to inseminate queens of the recessive line and propagate new colonies. The worker offspring from these colonies will segregate between colonies for the trait of interest in the approximate ratio of 1:1.

enough loci are scored, one or more will be physically linked to a gene(s) that influences heritable variation in the trait, which will be evident by a strong genotype–phenotype association at one or more marker loci (Fig. 3). The stronger the linkage and the effect of the gene on the phenotypic variation, the stronger will be this statistical association.

Since thousands of backcross workers can be easily produced, honey bees are particularly amenable to linkage mapping of traits expressed by, and easily scoreable in, individual workers. These include morphological and physiological traits, and some behavioural traits like sucrose threshold (Page *et al.*, 1998), age at foraging initiation (Rueppell *et al.*, 2004a), or engagement in specialist tasks like guarding (Arechavaleta-Velasco and

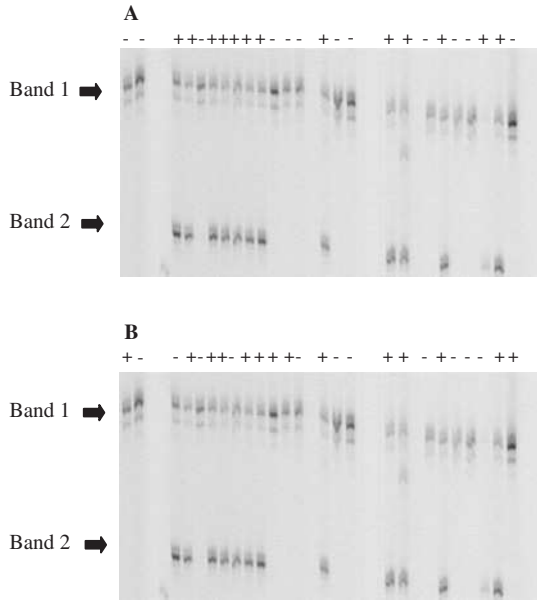


FIG. 3 An example of a test for genotype–phenotype association using microsatellite DNA markers. Statistical association between genotype (visualized through electrophoretic pattern – in this case, one band versus two) and binary phenotype (+, −) is the basis by which quantitative trait loci are initially identified. A strong association (as in A) suggests that variation in phenotype is influenced by a locus linked to this marker. An absence of association (as in B) indicates the marker is not linked to any gene of interest, and that additional makers need to be screened.

Hunt, 2004) (Table 1). An example of such a backcross is shown in Fig. 2A, in which alleles at an as-yet-unknown QTL are made to segregate among worker progeny of a single $F_1$ queen. The workers can then be scored for the phenotypic trait while being categorized into genotypic class. Any association between phenotype and genotype is indicative of the presence of a segregating QTL.

The mapping of colony-level, as opposed to individual-level, traits is more challenging, as Fig. 2B shows. Again, segregation occurs among the progeny of a single $F_1$ queen, but in this case the eggs remain unfertilized and develop into haploid males, which are then used to inseminate a large number of queens (~100) raised as virgins from the presumptive recessive parental line. In cases where the dominance relationship is unknown, two sets of backcrosses need to be performed, one from each parent. Here it is whole colonies that segregate for the behavioural trait, and thus it is the male parents of the colonies, not the offspring workers, that are genotyped for segregating QTLs. Obviously, colony-level traits will be more labour-intensive to map than traits expressed by workers, and for this

TABLE 1 Examples of genetically based task specialization in honey bees

| Behaviour | Description | References |
|---|---|---|
| Foraging behaviour | Preferred forage (pollen, nectar, water, or floral location) | Calderone and Page (1992); Calderone *et al.* (1989); Fewell and Page (1993); Guzmán-Novoa *et al.* (1994); Kryger *et al.* (2000); Oldroyd *et al.* (1991a,b, 1992, 1993); Robinson and Page (1989b) |
| | Age at onset of foraging | Calderone and Page (1988, 1991, 1996); Giray *et al.* (1999, 2000); Giray and Robinson (1994); Kolmes *et al.* (1989); Rothenbuhler and Page (1989) |
| | Scouting for food or nest sites | Dreller (1998); Robinson and Page (1989b) |
| Nest defence | Guarding | Breed *et al.* (2004); Giray *et al.* (2000); Robinson and Page (1988) |
| Brood care | Feeding and tending larvae | Page *et al.* (1989b); Robinson *et al.* (1990, 1994) |
| Interactions with other workers | Grooming other individuals | Frumhoff and Baker (1988) |
| | Feeding other individuals | Frumhoff and Baker (1988) |
| Nest homeostasis | Fanning in response to high temperatures | Jones *et al.* (2004) |
| | Corpse removal | Robinson and Page (1988) |
| Reproductive behaviour | Worker oviposition when queenless | Martin *et al.* (2004); Robinson *et al.* (1990) |
| | Worker reproduction when queenright | Châline *et al.* (2002); Montague and Oldroyd, (1998); Oldroyd *et al.* (1994) |

reason it is often difficult to achieve sufficient colony replicates to permit a mapping population of sufficient size to map QTL of even moderate effect.

About 350 evenly spaced genetic markers are required to generate a high-density linkage map of the honey bee, and most studies have phenotypic scores for at least 100 workers or colonies (Hunt *et al.*, 1995, 1998, 1999; Page *et al.*, 2000; Chandra *et al.*, 2001; Guzmán-Novoa *et al.*, 2002; Arechavaleta-Velasco and Hunt, 2003; Arechavaleta-Velasco *et al.*, 2003), though 300 is more desirable. The first honey bee maps were based on RAPD markers (Hunt and Page, 1995; Lapidge *et al.*, 2002), but these have been replaced by more repeatable and readily scoreable AFLP (Arechavaleta-Velasco and Hunt, 2004) and microsatellite (Solignac *et al.*, 2004) markers.

Statistical analysis of QTL data can become complex, and a detailed review is beyond the scope of this review. The reader is referred to texts such as Camp and Cox (2002) and Liu (1998) for authoritative descriptions. Nonetheless, we briefly present the principles here, so that readers unfamiliar with genomic analyses can appreciate the meaning of QTLs that have been reported as having an influence on honey bee behaviour, without having to delve into specialist literature.

To take the simplest analysis first, if a marker is genetically linked to a QTL of large effect (as in Fig. 3A), the QTL will reveal itself by a statistically significant difference in the mean phenotypic score of individuals that are of genotype $A_1A_1$ and individuals that are genotype $A_1A_2$ in backcross (cf. Fig. 2) progeny. Thus, a simple test for the presence of a QTL is to look for such loci by testing for genotype-phenotype association on a marker-by-marker basis. QTLs of large effect should lead to significant differences in the average phenotypic rank between genotypic classes. Because each locus is tested independently, however, there is no explicit reference to the linkage map, and because the number of marker loci examined is necessarily large there is a good chance that some loci will show spurious significance by type 1 error alone. For this reason, it is recommended that a stringent significance criterion be used in rank sum tests, such as $\alpha = 0.005$ or lower (Van Ooijen, 2004).

More sophisticated analyses will make use of a linkage map of the marker loci segregating in the cross. Software that seek an arrangement of loci that minimizes the number of crossovers required to explain the genotypes of backcross progeny are available for the production of such maps (e.g. Schiex and Gaspin, 1997). Having obtained a linkage map, one approach is to use 'interval mapping' (Lander and Botstein, 1989) to seek pairs of linked markers that show evidence of a QTL between them. For example, if we assume two linked marker loci, *A* and *B*, with a QTL between them, then we would expect the mean phenotypic scores of individuals of genotype $A_1A_2B_1B_1$ to be different to the mean phenotypic scores of individuals of genotype $A_1A_1B_1B_2$, depending on which marker, *A* or *B*, is closest to the QTL. The genomic region between markers *A* and *B* therefore designates an interval in which a QTL is located.

Interval mapping provides increased confidence about the presence of a QTL over single marker tests because all loci linked to the QTL should show a proportional association with phenotype, with more distant loci showing a smaller effect. Regression approaches to this procedure allow for more accurate estimation of the effects of a given putative QTL, in a multiple QTL model, by holding the effects of all other possible QTLs constant, and further allow the possibility of examining epistatic interactions between loci (Falconer and Mackay, 1996). This increases statistical power, reduces type 1 error, and can provide additional information about the genetic architecture of a trait. Such a multi-point mapping approach

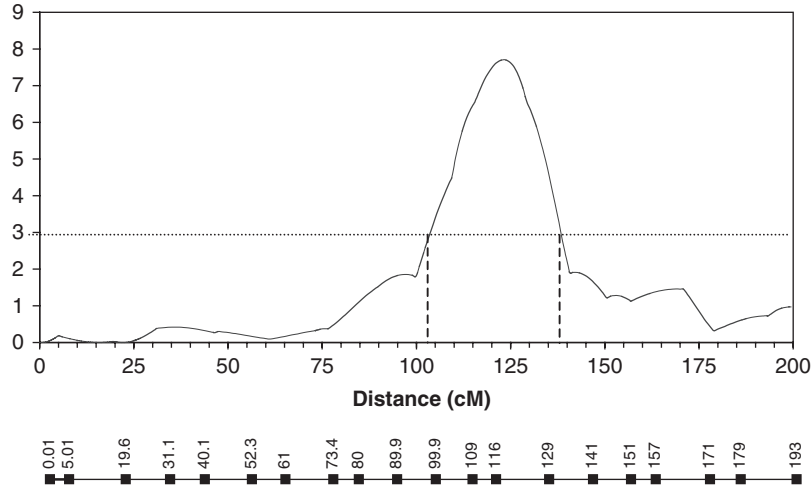


FIG. 4 A QTL profile. The LOD score (Y-axis) varies in magnitude over the linkage map (X-axis; in centiMorgans). LOD score peaks above the significance threshold (dotted line) indicate the presence of a segregating QTL. The genomic region corresponding to the area under the peak on the linkage map will contain candidate genes for the quantitative trait.

was used to great effect in the analysis of the interactions between the *pln* loci that describe variation in pollen hoarding behaviour among honey bee colonies (Rueppell *et al.*, 2004b).

QTLs are by convention presented as representations of linkage groups aligned against a continuous trace of the log-likelihood probability of the presence of a QTL (Fig. 4). The likelihood ratio of this hypothesis (presence of a QTL) and the null hypothesis (no QTL) taken at any point along the trace can be expressed as a standardized score called a LOD score. A LOD score therefore gives a statistical indication of the presence of a QTL at any point along the map. At map positions where the LOD score exceeds a pre-defined significance threshold, a QTL is declared. In practice, a minimum LOD score of between 1.5 and 3 can be significant, though a LOD score of 3 or higher is usually necessary to control for experiment-wise error at the 0.05 level in honey bee.

### 3.2.2 *From QTL to gene*

Finding a statistical association between a molecular genetic marker and a phenotype (Figs. 3 and 4) is a promising starting point. However, for a complete understanding of the genetic components of behaviour, we need to know the identity of the gene that affects the behaviour, what its product is, and how it is regulated. The advent of the honey bee genome project

(see the honey bee genome project and BeeBase web sites) has greatly facilitated the possibility of directly identifying honey bee behavioural genes. After detecting the location of a QTL, for example, candidate genes within its statistical boundaries can be identified by cross-referencing the linkage map to the honey bee genomic (actual sequence) map.

The general approach is to locate the DNA sequence closest to the QTL in the genome using BLAST software. A judgment must be made as to whether additional mapping is needed to narrow the search space. For example, if examination of the target genomic region contains an obvious microsatellite that was not included in the original screen, it may be advantageous to now include this marker and genotype the backcross progeny at this locus. Fine mapping by this and other means can more precisely define the QTL's statistical boundaries and location (Remington *et al.*, 2001).

A candidate gene may already be annotated – i.e. has previously been identified and the primary structure defined. Its molecular function may be known to some degree too, if membership to a recognized gene family can be inferred. It is possible that an annotated gene will have a function that suggests its involvement in the behaviour of interest. For example, a gene related to olfaction would be a strong candidate if the behaviour of interest relies on olfaction. It is at this stage where information regarding the natural history of the organism comes back into play. Which cues ellicit this behaviour? Which sensory mechanims are likely to be involved? What are the physiological processes that may be active? The answers to these types of questions can help narrow down a large list of candidate genes or help to identify a particularly promising subset of candidates. In any case, given that there will be a finite number of genes within a few centiMorgans of the QTL, it should be possible to at least identify all of the candidates, each the potential target of downstream functional analyses.

The candidate genes, once identified and annotated from the genomic database, can then be sequenced from the phenotyped individuals of the backcross and compared between alternate phenotypes. This comparative sequencing can help identify specific nucleotides that are statistically associated with behavioural polymorphism. These 'quantitative trait nucleotides' within QTLs could be the specific mutation that underlies the behavioural difference, or simply be linked to it. Because much allelic variation at the molecular level will be neutral or nearly so (Kimura, 1983), finding *the* difference again becomes a statistical pursuit. Obviously, it is difficult to conclude that a nucleotide polymorphism is functionally important by statistical association alone, but appropriate associative tests can be powerful in themselves (Anholt and Mackay, 2004) and, in combination with manipulative or comparative studies of gene expression, can be used to further develop or test a candidate gene's involvement. Nevertheless, genes that at this stage do not exhibit at least provisional

association at the nucleotide level with phenotypic variation in the trait are unlikely to be the primary source of genetic variation.

In addition to finding mutations via QTL screens and comparative sequencing, a quite separate screen could look for differences in gene expression, which may or may not be directly associated with an *a priori* detected mutation (Robinson and Ben-Shahar, 2002). Differences in gene expression can similarly be tested for association with behavioural polymorphisms, but in this case the source of variation is gene regulation rather than gene sequence. Genes whose expression can be shown to vary with the phenotype are candidate components in the molecular pathways that regulate the expression of the trait. In many cases, genes whose expression varies will be down-stream components whose expression is affected by a mutation in a regulatory up-stream component. Deconstructing these pathways is difficult, but as has been demonstrated in studies on immunity, for example (Evans *et al*., 2006), possible.

One platform available to screen for gene expression differences that are associated with a behavioural phenotype is quantitative reverse transcription PCR (qRT-PCR). In a qRT-PCR assay, the magnitude (i.e. fold-change) and direction (i.e. upregulated versus downregulated) of gene expression can be measured and tested for association with variation in phenotype. Ideally, if it can be demonstrated that mutations at single loci (detected by QTL or comparative sequencing) affect the phenotypic expression of a trait by changing the level of transcript abundance (detected by qRT-PCR) at that very same locus (e.g. Osborne *et al*., 1997), then this would strongly implicate the candidate gene as functionally important (Jansen and Nap, 2001; Doerge, 2002).

Further information on a candidate's involvement can be obtained from more manipulative experiments using, for example, gene expression interference technology, such as RNAi. If the behaviour can be experimentally altered by blocking the candidate transcript's expression, then a causal relationship between the gene and behaviour is demonstrated. RNAi technology shows great promise as a tool for better understanding the functional significance of individual genes in honey bees (Beye *et al*., 2002, 2003; Amdam *et al*., 2003b; Farooqui *et al*., 2004; Aronstein and Saldivar, 2005; Guidugli *et al*., 2005), however, the technique has not yet been widely employed in honey bee behavioural genetics.

## 4 Genetic architecture and task specialization

### 4.1 TASK STIMULUS AND TASK THRESHOLD

To understand honey bee behavioural genetics, we must first appreciate the genetic structure of colonies. A colony is neither an individual nor a

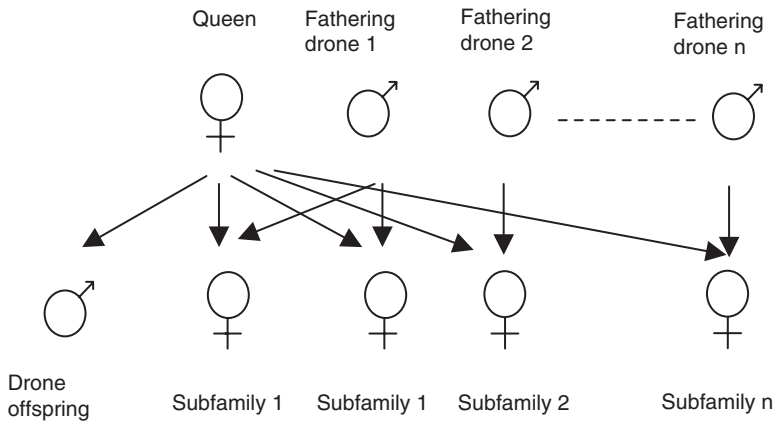


FIG. 5 The social and genetic structure of a honey bee colony. A single queen mates with multiple fathering drones to produce female (worker) offspring. The offspring sired by each drone represent different patrilines among the workers. Behavioural variation among patrilines can indicate that behavioural variation has a genetic basis. The queen will also lay unfertilized eggs parthenogenetically, which give rise to male (drone) offspring.

population, but an extended family (Fig. 5). Colony-level behavioural phenotypes arise primarily from the interactions of workers with each other and their environment (Page and Mitchell, 1998). Because queens mate with about 10–20 haploid males (Palmer and Oldroyd, 2000), the worker population comprises a mixture of full and half sisters (Page and Laidlaw, 1988). Further, because males produce sperm clonally, there is three times more genetic distance between subfamilies (or patrilines; daughters of different males) than there is among workers within them (Laidlaw and Page, 1984). This means that for any trait for which there is genetic variance for the probability that a worker will engage in a particular task (i.e. they differ in their *task threshold*), workers of different patrilines will behave differently in response to the same *task stimulus* (Fig. 6), some measure of the colony's need for a task is to be performed (Calderone and Page, 1988; Page *et al.*, 1989a; Robinson and Page, 1989a; Robinson, 1992; Bonabeau *et al.*, 1996, 1998;Theraulaz *et al.*, 1998; Beshers and Fewell, 2001; Fewell, 2003; Myerscough and Oldroyd, 2004). This results in the often-reported phenomenon of *task specialization*, in which workers of certain subfamilies are more likely to engage in particular tasks than workers of other subfamilies (Table 1), given a particular level of task stimulus.

Despite the widespread occurrence of task specialization, there is no simple relationship between a genetically based task threshold and actually performing that task or behaviour (Jones *et al.*, 2004; Graham *et al.*, 2006). This is because, within the context of a nest, the level of task stimulus experienced by individual workers is influenced by the activities of other workers (Page

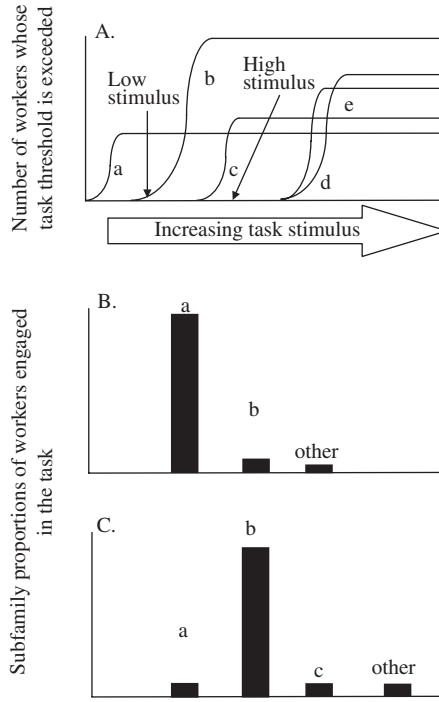


FIG. 6 A descriptive model of how differing task thresholds among patrilines translates into the number of workers engaged in a task. The model describes a situation in a small nest in which a few workers are available to engage in the task (i.e. they are of the correct age). (A) There is a genetic basis to task threshold such that patrilines (a,b,c,d,e) differ in their response to the same stimulus. (B) Patrilineal proportions in a sample of workers observed to be engaged in a task when the task stimulus is 'Low' (from A). At this level of stimulus, the task threshold of all members of patriline *a* is exceeded and all members of this patriline will engage in the task. A small number of patriline *b* will also be engaged, as may a small number of workers of other patrilines if their local stimulus is sufficiently high. (C) Here the stimulus is high enough that about 2/3 of the members of patriline *b* engage in the task. As this is a numerically dominant patriline, the proportion of workers of patriline *a* engaged in the task declines relative to the low stimulus scenario, even though the task threshold for all members of the patriline is exceeded. The task threshold model is subject to negative feedback.

*et al.*, 1989a; Calderone and Page, 1991, 1992; Myerscough and Oldroyd, 2004) and by unequal numerical strength or efficiency of patrilines. In Fig. 6 we assume a five-patriline colony in which the patrilines have a different task threshold for a particular task, like ventilating the nest entrance by wing fanning, for example. When the stimulus for fanning (e.g. concentration of $CO_2$ or nest temperature) is low, only the patriline with a low task threshold (*a* in Fig. 6) will engage in the task. When the task stimulus increases (e.g. when the ambient temperature is increased to 30 °C), workers of additional

patrilines (*b* and *c*) will engage in the task. If we sampled and genotyped the workers engaged in fanning over a range of temperatures, our model predicts that the patriline proportions in our sample would change. Importantly, however, there will not necessarily be a linear increase in the number of patrilines among fanning workers as the temperature is increased and the patriline with the lowest task threshold will not necessarily be the most represented at all levels of task stimulus. The reasons why this is so are complex and interacting. When a numerically large patriline starts to engage in a task, its efforts should reduce the level of stimulus within the colony. Thus the efforts of the numerically dominant subfamily will swamp a numerically smaller patriline of lower or equal task threshold. Furthermore, all workers have a diverse behavioural repertoire where each behaviour has its own threshold (O'Donnell and Foster, 2001; Weidenmüller, 2004) and workers may abandon one task should their threshold for another be exceeded.

### 4.2 BEHAVIOURAL OVERDOMINANCE

A related phenomenon to task specialization is *behavioural overdominance* (Moritz and Southwick, 1987; Hillesheim *et al*., 1989;Fuchs and Moritz, 1998). Behavioural overdominance describes the situation where a colony-level phenotype is strongly influenced by a relatively small proportion of workers. There are two main ways in which behavioural overdominace is likely to occur. First, if a colony's need for a task can be taken care of by a relatively small number of workers, who have a low threshold for that task, then the colony will have a colony-level phenotype that reflects that of the minority low-threshold workers (Fuchs and Moritz, 1998). Another way of looking at this is to note that a colony comprising a mixture of both low- and high-threshold workers for a particular task would have the same or similar colony-level phenotype as a colony comprised solely of low-threshold individuals (Arathi and Spivak, 2001). Second, behavioural overdominance can occur if the activities of a small number of workers lowers the task threshold of other workers or increases the task stimulus that nestmates perceive. Any colony-level phenotype that is pheromonally orchestrated is likely to show a behavioural overdominance, because low-threshold workers will release pheromones leading to a response in high-threshold workers that would not otherwise have responded to the stimulus (Guzmán-Novoa and Page, 1994; Hunt *et al*., 2003).

### 4.3 PHYSIOLOGICAL AND GENETIC BASIS OF TASK THRESHOLDS

The primary determinant of a worker's task threshold is its age (Lindauer, 1971; Seeley and Kolmes, 1991). Young workers perform tasks within the nest, but soon graduate to duties like guarding on the nest periphery (Calderone, 1998). At about three weeks of age, workers begin foraging

tasks that they undertake for the rest of their lives. This age-based task ontogeny is accompanied by fundamental changes in the exocrine and endocrine systems so that workers of a particular age are equipped with the appropriate glandular secretions that allow them to undertake tasks typical for that age. Thus, in young nurse bees the hypopharangeal glands are active, producing royal jelly for brood feeding. Later, the mandibular glands regress and the wax glands become active, allowing the worker to engage in comb building. At the onset of foraging, the wax glands will have regressed and the venom sack of the sting will be full. At this stage, the worker is motivated via neurochemical changes in its brain to forage and to learn the scents of flowers and their location (Bozic and Woodring, 1998; Schulz *et al*., 2002a).

The most fundamental change in a worker's adult life is the transition from nest-bound duties to foraging and the mechanisms behind this transition have been extensively studied. It is possible to manipulate age demography so that a colony is comprised mainly of nurse bees or of foragers (Robinson *et al*., 1989). As colonies require both nurses and foragers, manipulated colonies rapidly adapt by either accelerating or reversing the behavioural development of some of the workers, so that a more typical ratio of foragers and nurses is restored. The major conclusion of such experiments is that the rate at which a worker progresses from hive to foraging is modulated by social interactions with other workers (Robinson *et al*., 1992; Huang and Robinson, 1992, 1996, 1999; Giray *et al*., 1999; Jassim *et al*., 2000; Beshers *et al*., 2001). Foragers secrete a pheromone, ethyl oleate, which is transmitted via trophallaxis, and it is this pheromone that retards the development of nurse workers (Leoncini *et al*., 2004). In the absence of foragers, some workers become precocious foragers. Nurse workers have lower juvenile hormone (JH) titres than foragers, and progression to foraging tasks can be accelerated by treatment with JH (Fahrbach and Robinson, 1996; Giray and Robinson, 1996; Giray *et al*., 1999; Bozic and Woodring, 2000; Jassim *et al*., 2000; Sullivan *et al*., 2000, 2003; Bloch *et al*., 2001; Schulz *et al*., 2002a,b). Although workers that have had their corpora allata (sole source of JH) surgically removed still develop into foragers, the rate at which they do so is delayed (Sullivan *et al*., 2003). This suggests that JH is an important factor in regulating the behavioural development of workers.

Despite the important effects of the social environment on the rate of behavioural development, there is clear evidence that the age at first foraging (AFF) is genetically influenced (Giray *et al*., 1999; Rueppell *et al*., 2004a). Although we are unaware of any explicit demonstration, it seems likely that genetically based variance in the rate of progress through the normal age-based behavioural ontogeny would result in task specialization (Calderone and Page, 1988; Sullivan *et al*., 2000; Page and Peng, 2001; Page and Erber, 2002; Rueppell *et al*., 2004a). Patrilines with retarded development are more

likely to be found engaged in nest-based tasks than patrilines with rapid behavioural development. Thus, we suspect that it will be eventually shown that many instances of task specialization (Table 1) are actually emergent properties of genetically-based variance in rates of behavioural ontogeny. However, this is by no means the whole story as genetically-based variance in perception of the environment, and the degree of response to it are known to underpin most task specialization in honey bees.

In the next section we discuss examples where genetically-based differences in perception lead to differences in worker behavioural phenotypes.

#### 4.3.1 *Hygienic behaviour*

Hygienic behaviour can be quantified as a colony-level phenotype by the rate at which a colony cleans out brood cells containing dead larvae and dead pupae (Spivak and Gilliam, 1998a,b). Hygienic behaviour can be experimentally assayed by providing a colony with a small piece of dead brood, usually 100 brood cells. Colonies that remove all the dead pupae within 48 h are said to be 'hygienic'. Colonies that retain dead brood for more than 48 h are, by contrast, said to be 'non-hygienic' (Spivak and Downey, 1998). Hygienic behaviour is an important commercial trait because hygienic colonies are resistant to brood diseases and parasites (Spivak and Gilliam, 1998a,b; Spivak and Reuter, 1998, 2001). Genetic markers that could identify strongly hygienic colonies in the field would be very useful as a tool for selecting choice colonies for breeding and avoiding colonies that do not carry the hygienic marker.

For a colony to express the hygienic phenotype, a proportion of its middle-aged (2–3 weeks old) workers must sense the dead brood, uncap the brood cell and remove the infected pupae for disposal outside the nest. In his now classic study of behavioural genetics, Rothenbuhler (1964) proposed that the two steps of this process, uncapping and removal, are under separate genetic control, each behaviour controlled by a single unlinked Mendelian locus. This hypothesis has been very useful for understanding the behaviour and promoting new tests. The two-locus model is, however, probably an over-simplification. Moritz (1988) suggested that Rothenbuhler's data was more suggestive of three loci than of two, and Lapidge *et al*. (2002) suggested that the trait showed a quantitative pattern of inheritance, potentially involving many loci. Nonetheless, all authors agree that there is a strong genetic component to hygienic behaviour and that variation in this two-step task is controlled by a small number of loci affecting the hygienic threshold of workers.

Hygienic behaviour is based, in part, on the stimulus threshold of reaction to the odours of disease-killed brood. In a line bred for increased hygienic behaviour, workers can discriminate between, and respond to, the odours of healthy and disease-killed brood at lower concentrations than

can workers from a line bred for poor hygienic behaviour (Masterman *et al*., 2001). When exposed to the same level of odour stimulus, hygienic-strain bees experience a stronger electrical signal in the antennal lobes of their brain, generating a higher level of the neuromodulator octopamine than do non-hygienic bees exposed to the same level of stimulus (Masterman *et al*., 2001; Spivak *et al*., 2003). High levels of octopamine appear to be a crucial factor behind a wide range of behaviour in honey bees, including hygienic behaviour, possibly because it is necessary for the formation of olfactory memory (Farooqui *et al*., 2003).

It is as yet unclear how the differing stimulus threshold between hygienic and non-hygienic strains is set. Is it that hygienic strain workers have more odorant receptors than non-hygienic strains, or do the same number of receptors result in a stronger signal that results in greater release of octopamine? We do not yet know the mechanism.

### 4.3.2 *Foraging Specialization*

Honey bee foragers show genetically-based specialization for water, nectar, or pollen collection, as evidenced by some patrilines that are more likely to collect pollen, nectar or water than others (Table 1). There is a negative correlation between the size of nectar loads and pollen loads carried by workers (Hunt *et al*., 1995; Page *et al*., 2000; Rueppell *et al*., 2004b), and this suggests that foragers are constrained by a maximum loading such that they cannot fly efficiently with both a fully-laden crop and corbicula (Feuerbacher *et al*., 2003) – hence the tendency for specialization on food types when on foraging trips.

The tendency for a worker to forage for water, nectar or pollen is strongly predicted by its sucrose threshold – the concentration of sucrose that a worker can distinguish from water in a proboscis extension response test (PER test, see Box 1) (Page *et al*., 1998; Scheiner *et al*., 2004). Water foragers sampled at the nest entrance have the lowest sucrose threshold. Those returning with pollen have the next highest, and those returning with nectar have the next highest response (Page *et al*., 1998; Pankiw and Page, 2000; Scheiner *et al*. , 2001, 2003a). Dreller (1998) found a strong genetic component to scouting behaviour. Hence, because individuals with the highest sucrose threshold are the most likely to return without any load, it may be that these workers are the ones scouting for very high-quality nectar sources.

Day-old workers that have not yet been exposed to environmental cues of the colony's nutritional needs show markedly different sucrose response thresholds, and these thresholds strongly predict whether they will become water, pollen, or nectar foragers later in life (Pankiw and Page, 1999, 2000). Thus it appears that a worker's innate sucrose response threshold has a strong genetic component, and this apparently leads to frequently reported specialization in foraging tasks (Table 1).

Box 1 – PER Test showing individual worker bees being presented with a droplet of sucrose. (Photo Courtesy of R. Maleszka)

In the proboscis extension response test, a bee is confined in a narrow tube with its head emerging from one end. To test the bee's response to sucrose, its antenna is touched with a small drop of sucrose in solution. If the bee extends its tongue, it means that it has responded (Kuwabara, 1957). The PER test can be used in a variety of contexts for behavioural research. First, it can be used to test the sucrose concentration that a worker can distinguish from water (Page *et al.*, 1998). Presumably, this is a measure of the individual's sucrose threshold. Second, it can be used as an assay for a worker's ability to learn (Bitterman, 1996; Chandra *et al.*, 2000, 2001; Ferguson *et al.*, 2001). The bee is first exposed to a conditioning stimulus, usually an odour, which is immediately followed by touching a small drop of sucrose to the antenna. If the bee extends its tongue, then it is allowed to feed from the sucrose. Most bees will learn to associate the odour with the reward after just one entrainment. Third, the test can be used to determine the relative ease with which workers can learn to distinguish odours (Masterman *et al.*, 2000).

The central role of sucrose threshold response in determining specialized foraging is not surprising, given that sucrose is the major carbohydrate source for honey bees, requiring that they be able to assess the concentration of sucrose solutions. The antennae and proboscis are replete with sucrose receptors that have direct connections to areas of the brain associated with memory formation and reward mediated via the release of octopamine (Menzel and Müller, 1996; Hammer and Menzel, 1998). Sucrose rewards are apparently important, if not essential, in the formation of memory about foraging tasks (Menzel, 1999; Scheiner *et al*., 2003b) and bees with a low sucrose threshold can learn more easily in response to a sucrose reward than individuals with a high sucrose threshold (Scheiner *et al*., 2001). Interestingly, three QTLs for the ability to associate an odour with a sucrose reward have been identified (Chandra *et al*., 2001), but we do not know how this phenomenon relates to sucrose response threshold.

Although born with an innate sucrose threshold, a worker's threshold is modulated throughout life by environmental factors related to the colony's nutritional needs. This is necessary because the sucrose threshold of the colony members is tuned such that they respond appropriately to the colony's need (for pollen or nectar) and to the floral sources available. If colonies are foraging on nectar sources of high sucrose concentration, the sucrose threshold of workers is raised (Pankiw *et al*., 2004), reducing the probability of bees foraging at low sucrose concentration forage patches or dance for them (Seeley, 1986; Seeley *et al*., 1991, 2000). Presumably this shifts some workers of intermediate threshold towards pollen foraging. Furthermore, if colonies have a large number of larvae to feed, pheromones produced by the brood lower the sucrose threshold, increasing the probability that foragers will collect pollen thereby reducing the average age of workers at onset of foraging (Eckert *et al*., 1994; Pankiw *et al*., 1998, 2002; Dreller *et al*., 1999; Pankiw and Page, 2001, 2003).

Foragers can also directly detect the amount of pollen stored in their colony (Dreller *et al*., 1999; Dreller and Tarpy, 2000; Vaughan and Calderone, 2002). Depletion of stored pollen results in an increase in colony-level foraging, arising from an increase in the proportion of workers engaged in pollen foraging, the number of trips individuals make, and the size of the loads of pollen that they carry (Fewell and Winston, 1992; Fewell and Bertram, 1999; Janmaat and Winston, 2000; Rotjan *et al*., 2002). As yet, there has been no demonstration that the sucrose thresholds of foragers are raised in pollen-deprived colonies, but this is a clear prediction if sucrose response threshold is the prime mechanism promoting foraging specialization. The sucrose threshold increases with age, meaning that younger foragers are more likely to collect pollen than older ones (Rani and Jain, 1997; Pankiw and Page, 1999).

The above remarks indicate that sucrose response threshold is a significant causal factor influencing foraging task specialization (Fig. 7).

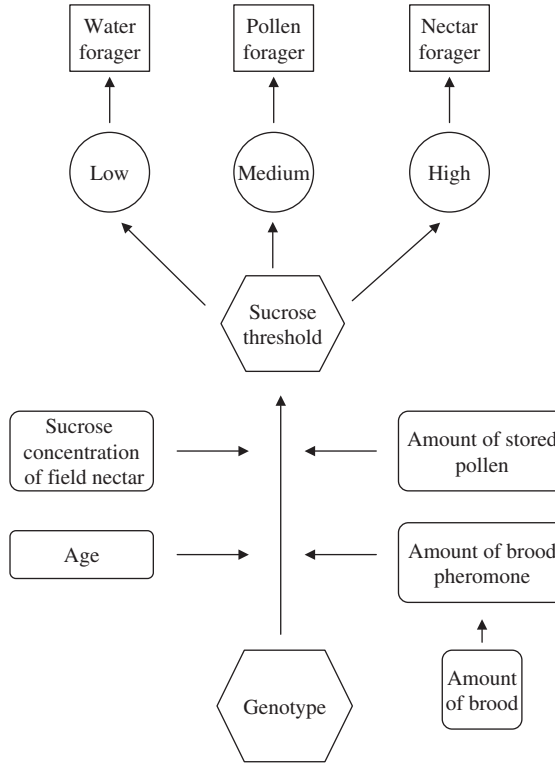


FIG. 7 Hypothetical relationship between factors affecting sucrose threshold, which in turn affects the tendency of individual workers to perform certain tasks related to foraging. Individuals with a low threshold will tend to forage for water, while those with a medium threshold will have a tendency to forage for pollen, and individuals with a high sucrose threshold response tend to forage for nectar.

Physiological mechanisms behind the genetic influence on sucrose threshold are easy to envisage, and could include the number of sucrose receptors on the mouthparts and antennae, and the extent of octopamine release after stimulus by sucrose (Barron *et al.*, 2002; Schulz *et al.*, 2002a).

Four significant QTLs influencing foraging specialization have been identified, each of which is genetically linked to loci that affect foraging behaviour in individual workers. Three of these loci, *pln1*, *pln2,* and *pln3*, were identified via linkage mapping of workers segregating in backcrosses between lines selected at the colony level for high and low pollen hoarding (as in Fig. 2) using neutral molecular markers (Hunt *et al.*, 1995). The *pln* loci have been identified in different crosses and populations, suggesting that they may be a general attribute of honey bees, potentially present in all populations (Hunt *et al.*, 1995; Page *et al.*, 2000; Rueppell *et al.*, 2004b).

Other loci, such as *Amfor*, the *Apis* orthologue of *Drosophila's forager* gene and encoding cGMP-dependent protein kinase (Ben-Shahar *et al.*, 2003), and *Ammvl*, the *Apis* orthologue of *Drosophila*'s *malvolio* (Ben-Shahar *et al.*, 2004), either directly influence the tendency to forage for pollen or nectar, or are linked to such a locus (Rueppell *et al.*, 2004b). Owing to the possibility that *Amfor* and *Ammvl* are not directly involved in foraging specialization, it is best to provisionally regard *Amfor* and *Ammvl* as candidate QTLs for foraging specialization rather than as genes causing behavioural variation.

The four foraging specialization loci, i.e. *pln1-3* and *Amfor (pln4*), interact epistatically to produce an individual's composite genotype that strongly influences the kind of forage that a worker is likely to collect. The actual genes associated with loci are yet to be identified, and so we do not know if or how these genes causally influence the sucrose threshold of workers. However, because these loci were first mapped in a cross between strains that show low and high sucrose thresholds (Page *et al.*, 1998; Pankiw and Page, 1999), such a relationship is possible. It would be interesting to see if these loci influence sucrose threshold, and thus establish a clear pathway from gene, through physiology (sucrose threshold) to behaviour (foraging specialism) (Fig. 7). Rueppell *et al.* (2006) recently identified an additional QTL for the sucrose response threshold itself.

*4.3.2.1 Amfor.* One of the most clear-cut examples of a single gene effect on natural variance in behaviour is the forager gene of *Drosophila* (Pereira and Sokolowski, 1993; Sokolowski, 2001). Two alleles exist in natural populations. The *for*R allele has a frequency of about 70% (Sokolowski *et al.*, 1997). Individuals carrying the *for*R allele are of the "rover" phenotype and forage over a larger area than individuals that are homozygous for the "sitter" allele *for*S (at 30% frequency), which are more sedentary (Debelle and Sokolowski, 1987). Forager encodes a cyclic guanosine monophosphate (cGMP) dependent protein kinase (PKG), and rovers have a much higher expression of the gene and PKG in their brains (Osborne *et al.*, 1997). Because of its effects on foraging behaviour, forager was investigated as a candidate gene influencing foraging behaviour in honey bees (Ben-Shahar *et al.*, 2002; Ben-Shahar, 2005; Fitzpatrick *et al.*, 2005).

The honey bee orthologue, *Amfor*, shows increased expression, and PKG activity levels are much higher in workers engaged in tasks outside the nest (Ben-Shahar *et al.*, 2003). Treatment of young workers with cGMP leads to precocious foraging and an increase in phototaxis, though the treatment itself may be partially causal. This led to the interpretation that in honey bees, cGMP initiates phototaxis, drawing middle-aged bees to the nest entrance where they are stimulated to forage by the smells and dances of older foragers (Ben-Shahar *et al.*, 2003). However, this indirect mechanism seems an unlikely hypothesis because many species of honey

bees nest in the open, where all the workers are exposed to constant light (Oldroyd and Wongsiri, 2006). Rather, it may be that cGMP stimulates foraging behaviour directly, possibly even to the level of foraging specialization (Rueppell *et al*., 2004b).

### 4.3.3 *Nest defence*

Honey bee nest defence is an extremely complex colony-level character (Collins *et al*., 1980; Breed *et al*., 2004). Most authors recognize two distinct components of colony defence: guarding and stinging. In any colony, some (5–10) or many ( > 100) workers will stand near the entrance and act as guards (Breed *et al*., 1992). The guards adopt a characteristic posture, with their front legs held off the substrate, their mandibles apart, and wings slightly spread (Butler and Free, 1952; Ghent and Gary, 1962). The guards often approach returning foragers, antennating them, and assessing whether they are non-nestmates. Should a non-nestmate attempt to enter the colony, the guard may grasp the intruder and eject it (Ribbands, 1954; Breed *et al*., 1992; Downs and Ratnieks, 1999).

Stinging behaviour occurs when a colony is disturbed by a predator and workers leave the nest to sting it. The guards are important to the initiation and coordination of stinging. If a predator approaches a colony, the guards will fly out to attack it, buzzing around the intruder, and possibly biting, and stinging. If the intruder does not withdraw, the guards at the nest entrance release alarm pheromones by exposing their stings. These pheromones, primarily isopentyl acetate (Boch *et al*., 1962), alert nestmates, many of which will join the fray (Arechavaleta-Velasco and Hunt, 2003).

Stinging behaviour is generally quantified by measuring the time to first sting after a stimulus, the number of stings in a target provided near the colony under test, and some measure of the duration of attack after the stimulus is removed (Stort, 1974; Collins and Kubasek, 1982; Breed, 1991). Sting stimuli include physical disturbances, exposure to alarm pheromone, or movement – workers tend to sting things that move. Honey bee colonies vary greatly in their stinging behaviour (deGrandi-Hoffman *et al*., 1998) and defensiveness has a strong genetic component (Collins *et al*., 1984; Breed and Rogers, 1991; Stort and Gonçalves, 1991; Guzmán-Novoa *et al*., 1999). In particular, the tropically-adapted African *A. m. scutellata* and its New World derivatives are much more defensive than typical ecotypes of European origin (Collins *et al*., 1982; Guzmán-Novoa and Page, 1993; deGrandi-Hoffman *et al*., 1998). Africanized bees react to a visual stimulus 20-times faster than European bees, and deposit eight-times as many stings in experimental targets (Collins *et al*., 1982).

Guarding behaviour is a good example of task specialization – within a colony, guard bees are drawn from a non-random set of patrilines (Robinson and Page, 1988). The basis of this specialization appears to arise

not from differing probabilities of engaging in guarding behaviour, but from the number of days in which individuals engage in the behaviour (Moore *et al.*, 1987; Arechavaleta-Velasco and Hunt, 2003; Hunt *et al.*, 2003). Most guards engage in the activity for about two days, but some individuals persist for six days (Moore *et al.*, 1987; Breed *et al.*, 1989; Arechavaleta-Velasco and Hunt, 2003). Clearly, the duration of guarding behaviour can lead to task specialization, even if there is no genetic influence on the probability of engaging in guarding.

There is a strong correlation between the persistence of individual guards and colony stinging behaviour (Breed *et al.*, 1989). This suggests a causal link between the number of guards and colony level stinging behaviour. More guards mean that the colony is more likely to notice an intruder, decreasing the time to attack. A larger number of guards will also increase the amount of alarm pheromone released when a colony is disturbed, thus increasing the ferocity of the attack. Therefore, genetically-based variance in the number of days an individual spends guarding results in both task specialization in guarding and colony-level variation in stinging. We note that delay in transition from guarding to foraging, or a precocious transition from nursing to guarding could both produce this effect, supporting the hypothesis that genetically-based variance in the age at which behavioural transitions occur plays a pivotal role in the organization of work in honey bee colonies (Page *et al.*, 1991; Huang and Robinson, 1992, 1996; Huang *et al.*, 1994; Fahrbach and Robinson, 1996; Trumbo *et al.*, 1997; Robinson and Huang, 1998; Giray *et al.*, 1999; Schulz and Robinson, 1999; Jassim *et al.*, 2000; Sullivan *et al.*, 2000; Leoncini *et al.*, 2004).

Hunt *et al.* (1998) identified five potential QTLs (designated *sting1* – *sting5*) that are apparently involved in the degree of stinging behaviour exhibited by colonies. The existence of *sting-1*, *sting-2* and *sting-3* was subsequently confirmed in independent crosses (Guzmán-Novoa *et al.*, 2002; Arechavaleta-Velasco *et al.*, 2003; Arechavaleta-Velasco and Hunt, 2004). *Sting-1* influences both the degree of colony-level stinging behaviour (time to first sting) and the probability of being a guard. The tendency to sting is apparently the dominant allele at this locus (Guzmán-Novoa *et al.*, 2002), but no candidate gene has been identified. *Sting-2* and *sting-3* affect the probability of guarding only (Arechavaleta-Velasco *et al.*, 2003), and a further eight loci may also affect this trait (Arechavaleta-Velasco and Hunt, 2004).

The *sting-2* locus has a total of 15 predicted genes within an 81 Kb BAC clone containing an STS linked to *sting-2*. This region does not include the whole confidence interval delimited by the QTL, so may not contain the causal gene(s). However, a large proportion of these predicted genes appear to be transcribed and are unique to honey bees (Lobo *et al.*, 2003). One of these transcripts shows a number of base substitutions between European and African samples, and is therefore a strong candidate to be the gene linked to the QTL marker. If confirmed, this would be the first

example of a gene that influences self-sacrificing task specialization being identified via linkage mapping in the honey bee.

The defensive response of a honey bee colony is largely mediated by alarm pheromones (Breed *et al*., 2004). Thus, in addition to the number and persistence of guards, the amount of alarm pheromone produced by individual workers and the reaction to these pheromones by other bees is likely to be important to the defensiveness of a colony. There is quantitative genetic variation for both the production of alarm pheromones (Collins *et al.*, 1987a) and for responses to it (Collins *et al.*, 1987b), and these two QTLs are not linked. A number of additional QTLs associated with production of the major sting-produced alarm pheromones have been mapped (Hunt *et al.*, 1999), but these await confirmation in independent crosses.

#### 4.3.4 *Dance communication*

Upon returning from a foraging trip, a successful forager may perform a communication dance that alerts her nestmates to the presence and location of her profitable patch (von Frisch, 1967). The dance is a stylized re-enactment of the foraging trip. During the 'waggle phase' of the dance, the worker strides forward while vigorously vibrating her abdomen from side to side (Tautz *et al.*, 1996). The alignment of the bee's body during the waggle indicates the direction of the profitable patch. If the bee is aligned straight up the comb during the waggle run, the dancer is indicating a patch that is directly in the direction of the sun's current azimuth (the point where the sun is over the horizon). If the waggle run is aligned straight down the comb, the dance indicates a patch that is precisely opposite the sun's azimuth. And a dance orientated at three o'clock indicates a patch at 90° to the current azimuth. We will return to the directional aspects of the bee's dance in the next section.

The duration of the waggle run indicates the distance to the goal; short runs indicate nearby targets and prolonged runs indicate distant ones (von Frisch, 1967). Thus the bees that follow the dance can estimate the distance to the target food source by determining the average duration of the waggle runs. As the target gets closer to the colony, the dance becomes more and more hurried. At some point, the dancer is unable to complete the regular figure-eight that characterizes the true waggle dance and tends to run in excited circles, sometimes wagging her abdomen and sometimes not (Beekman *et al.*, 2005). This form of dance is known as a 'round dance' (von Frisch, 1967). Early studies suggested that the slope of the curves that relate distance to dance tempo (Fig. 8) differ according to ecotype (Boch, 1957; von Frisch, 1967) and species (Lindauer, 1956, 1957; Punchihewa *et al.*, 1985). More recent experiments have not been able to confirm differing species-specific dance forms (Dyer and Seeley, 1991; Sen Sarma *et al.*, 2004).

Furthermore, we now know that the flying bee perceives the distance that she has traveled as the amount of optic flow past her eye as she flies

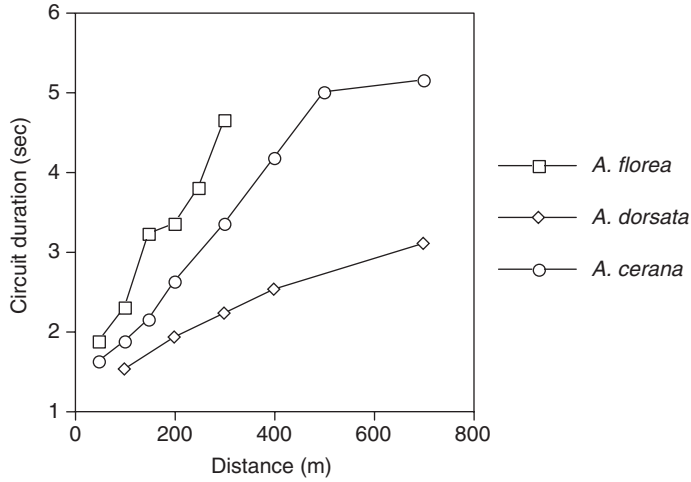


FIG. 8 Dance tempo. The duration of each dance circuit, or 'tempo', varies with the distance to the food target. More distant goals result in more ponderous dances. The relationship between tempo and distance is claimed to be variable between species within the genus *Apis*. Redrawn from Lindauer (1971).

along (Srinivasen *et al.*, 2000; Esch *et al.*, 2001). Therefore, the visual texture of the environment in which the bee is flying must be important to the slope of the dance curve, and studies done on different species/ecotypes in different environments confound genotypic effects with environmental effects. Thus, the relative importance of genetics and environment in shaping the dance tempo curves is currently unresolved. Nonetheless, backcross experiments controlling for environment suggest that the foraging distance at which foragers switch from waggle to round dance has a genetic component (Rinderer and Beaman, 1995; Johnson *et al.*, 2002).

## 5 Endogenous clocks

### 5.1 CIRCADIAN RHYTHMS

As flowers do not secrete nectar uniformly throughout the day (Beutler, 1930), honey bees live by the clock. Foragers readily learn to visit flowers or an artificial feeder only when nectar is available (Beling, 1929; Wahl, 1932; Visscher and Seeley, 1982; Schneider and McNally, 1993). Furthermore, time is crucial to the honey bee's navigation system. Bees use the current position of the sun's azimuth as an arbitrary reference point from which direction is measured, both when performing communication dances and when flying to and from their feeding place (von Frisch, 1967). Unlike the human arbitrary directional reference (north), the bee's reference

moves during the course of the day, and therefore requires constant time-compensated calibration. Foragers learn the movements of the sun at their location, and can use this learned trajectory to make predictions about the sun's current position, even if they cannot see it (von Frisch and Lindauer, 1954; Lindauer, 1960). For example, a bee that dances for prolonged periods deep within the dark interior of her nest makes adjustments to the angle of her waggle run to compensate for the sun's movement, even during the period of her dance (Lindauer, 1971).

The ability of foragers to track the time of day and position of the sun suggests that they have an endogenous circadian clock, or *Zeitgedächtnis* (Renner, 1955, 1957; Spangler, 1973; Moore and Rankin, 1985; Frisch and Ascoff, 1987). Young workers are arrhythmic (Spangler, 1972). They perform in-hive duties at any time of day or night. However, as they grow older, their patterns of activity become more rhythmic and based on a 24 h cycle (Crailsheim *et al.*, 1996; Moore *et al.*, 1998; Toma *et al.*, 2000; Bloch *et al.*, 2001). As mentioned above, some genotypes make the transition to foraging at an earlier age than other genotypes, and genotypes with an early onset of foraging are also the first to develop rhythmicity (Giray and Robinson, 1994; Bloch *et al.*, 2002). However, treatment with JH, which accelerates behavioural development, does not accelerate rhythmicity (Bloch *et al.*, 2002).

The molecular-genetic basis of endogenous rhythms has been elucidated for a variety of biological systems, particularly in *Drosophila*. Briefly, diurnal behaviours of the fly, such as mating and feeding times, are calibrated by an endogenous molecular clock. The primary oscillator of the clock arises from the interaction of two genes: *Period* (*Per*) and *Timeless* (*Tim*). The gene products of *Per* and *Tim* interact in the cytoplasm to produce an unstable PER-TIM heterodimer. This heterodimer then enters the nucleus and shuts down the transcription of *Per*. As the unstable PER-TIM heterodimer decays, the transcription of *Per* commences anew and the cycle repeats itself. The system can be entrained because TIM is light-sensitive and the PER-TIM heterodimer cannot accumulate in low light (Price *et al.*, 1995).

The study of the molecular basis of circadian rhythm in honey bees is still in its infancy, but mechanisms are likely to be similar to those that have been elucidated in other insects. *Apis* orthologues of *Per* oscillate in their expression diurnally in both *A. mellifera* (Toma *et al.*, 2000; Bloch *et al.*, 2003) and *A. cerana* (Shimizu *et al.*, 2001). The *A. mellifera* orthologue, *AmPer*, has 55% amino acid similarity to *Per* of *D. melanogaster* (Toma *et al.*, 2000). It is expressed in a particular region of the brain, suggesting that this region acts as the neurological clock. As one might expect, *Per* expression is high in rhythmic foragers and low in arrhythmic nurses (Toma *et al.*, 2000; Bloch *et al.*, 2001).

It is important that studies on the honey bee molecular clock are continued because time is central to so much of the honey bees' behaviour. In addition to many aspects of navigation, honey bee mating times are almost certainly

controlled by the circadian clock. Honey bees mate on the wing. To avoid the possibility of inter-specific matings, each species has a species-specific mating time (Oldroyd and Wongsiri, 2006). For example, in Chanthaburi, south eastern Thailand (Rinderer *et al.*, 1993), drones of *A. andreniformis* commence mating flights close to the sun's zenith and finish by 13:45 h, *A. florea* from 14:00 h to 16:45 h, *A. cerana* from 15:15 h to 17:30 h, and *A. dorsata* from dusk to 18:45 h. It is most unlikely that drones use the position of the sun directly to determine the time of day that they should fly. In cavity-nesting species (*A. mellifera*, *A. cerana*, *A. nuluensis*, *A. koschevnikovi* and *A. nigrocincta*). The drones cannot see the sun when they are inside their hive; and even in open-nesting species (*A. andreniformis*, *A. florea*, *A. dorsata* and *A. laboriosa*) drones often don't have a direct view of the sun. Thus, it would seem likely that they use their endogenous clock to determine the time that they should fly. Interestingly, the same species show different mating times in different localities (Koeniger and Koeniger, 1991; Oldroyd and Wongsiri, 2006) and this gives the opportunity to elucidate the connections between the molecular genetic oscillator and behaviour – drone flight time.

### 5.2 ULTRADIAN RHYTHMS

Much of the honey bee's behaviour is based on cycles with a phase several orders of magnitude shorter than the circadian clock based on the cyclic expression of *Amper*. Vibration of the abdomen during the waggle dance, for example, seems more likely to be controlled by a neurological oscillator than a molecular genetic one. And, yet, some molecular genetic oscillators have cycles of less than a minute, including defecation behaviour in the nematode *Caenorhabditis elegans* (Iwasaki and Thomas, 1997; Take-uchi *et al.*, 2005). Further, some *per* mutants alter the frequency of the courtship song of *D. melanogaster* males (Konopka *et al.*, 1996), suggesting that *per*-like genes may play a general role in setting some biological rhythms, even those of very short periodicity. If so, clock genes would be good candidates for the control of the dance language.

## 6 Field and selection techniques

### 6.1 BREEDING AND SELECTING FOR COLONY-LEVEL TRAITS

Unlike all other livestock, the productivity of honey bee colonies does not depend primarily on individual physiological traits, like growth rate, but on social traits, like honey hoarding and defensiveness. Even a trait like disease resistance is strongly influenced by social factors (e.g. hygienic behaviour) (Evans *et al.*, 2006), as well as by the individual's innate immune system. Thus, when beekeepers seek to genetically improve honey bee

stocks they are primarily interested in improving colony-level behavioural traits, rather than physiological ones of individual workers.

The genetic improvement of honey bees for commercial beekeeping is beset with problems. As mentioned in the Introduction, because of the genetic load imposed by the sex locus, the effects of inbreeding are severe in honey bees. Therefore, the design of a successful selection program must minimize the rate of inbreeding (Page and Laidlaw, 1982; Ruttner, 1988; Ebbersten, 1996; Laidlaw and Page, 1997). One breeding design that is useful in making genetic progress and minimizing the effects of inbreeding is shown in Fig. 9. In this design, a series of breeding lines is maintained. Each year the best queen within each line is used to be the mother of the next generation. This queen also contributes drones to a pooled

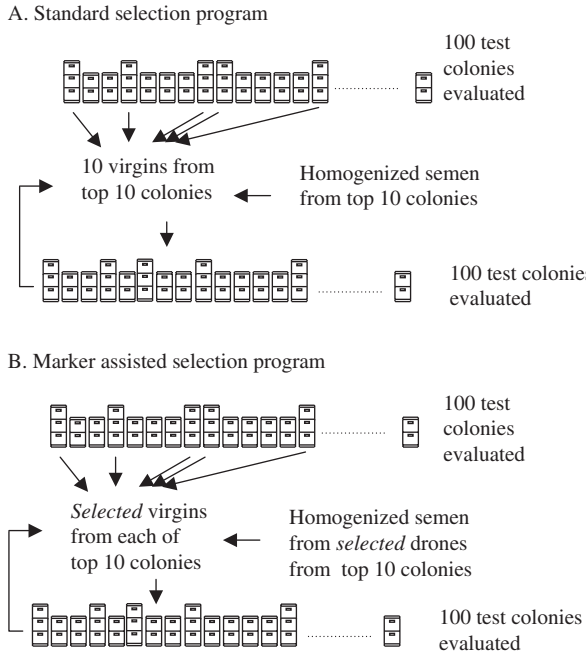


FIG. 9 Selection programmes for colony-level traits in honey bees. (A) The standard selection programme whereby, each year, colonies are evaluated for favourable traits (e.g. honey production, docility, hygiene, etc). Queens are then reared from only the single best colony in each of ten lines. The top-ten queens are inseminated with homogenized pooled semen – a diversity-generating step is the key defence against inbreeding. Drones for the inseminations are obtained from all of the selected colonies. (B) Marker-assisted selection programme. We propose that 20 virgin queens should be raised from each of the ten lines. A wing clip could then be taken from all virgins when they are a day old and DNA extracted from these clippings, without any harm to the queens. Only those queens that carry favourable alleles at the marker loci would then be inseminated with the pooled semen, and not the rest.

population, from which semen is collected that is then used to inseminate all of the queens in the programme. Methods for insemination using pooled centrifuged semen are reviewed by Harbo (1986, 1990).

### 6.2 MARKER-ASSISTED SELECTION

The usual selection criteria for commercial breeding programs are honey production, disposition (i.e. low nest defence), and perhaps disease resistance. Recent advances in the behavioural genetics of bees should soon see the development of genetic markers for various traits, particularly hygienic behaviour (Lapidge *et al.*, 2002) and stinging behaviour (Lobo *et al.*, 2003). This provides the possibility for enhancing the effectiveness of selection programmes via the use of marker-assisted selection. When producing daughter queens from the selected queen, marker-assisted selection will allow the breeder to chose from among the virgin daughters those that carry the preferred allele at those loci for which information is available. For example, 20 virgin queens could be raised from each of the breeding lines (Fig. 9). A wing clip could be taken from all virgins when they are a day old, and DNA extracted from these clippings, without any harm to the queens (Châline *et al.*, 2004; Gregory and Rinderer, 2004). Those queens carrying favourable alleles at marker loci for stinging and hygienic behaviour could be inseminated with the pooled semen and the rest discarded. Such a programme would increase the strength of selection, while reducing the number of colonies evaluated in expensive field evaluations. Marker-assisted selection would be particularly useful when screening for new genetic material from outside a closed population.

Marker-assisted selection of queens seems reasonably straightforward, but selection of drones is more difficult. As a large number of drones of the right age (optimally older than 16 days) are required for the inseminations, they are often in short supply. In principle, one could individually mark a large number of drones when they are a few days old, take a wing clip and genotype them as with virgin queens. After genotyping and when the drones are sexually mature, only those individuals carrying the preferred allele would be used in inseminations.

## 7 Social behaviour

### 7.1 DEFINING SOCIAL AND EUSOCIAL TRAITS

If social behaviour is recognized as simply involving communication and cooperation, then because honey bees live in colonies a great deal of their behaviour will be subsumed by this definition. Indeed, few bee behavioural traits could be truly excluded from the social label. Certain traits will,

however, be more directly related to honey bee reproductive division of labour and their caste system. These traits can more readily be defined as truly social, or eusocial. Eusocial characters include those affecting reproductive altruism and are likely to have evolved to some extent via indirect selection (Hamilton, 1996). Altruistic traits may also have characteristic genetic architectures or patterns of expression that differ from traits that are directly selected (Queller and Strassmann, 1998), and are thus of very great interest to sociobiologists.

## 7.2 THE GENOMICS OF EUSOCIAL TRAITS

The three traits most widely regarded as defining eusociality are cooperative brood care, overlap of generations, and a reproductive division of labour, whereby some individuals forego their own reproductive output in order to help others reproduce (Wilson, 2000). Therefore, studies that attempt to uncover the genetic basis for variation in any one of these three traits could be considered 'eusociogenomic'. Most interesting, however, will be studies on reproductive division of labour, and thus target the genetic basis for variation in reproductive altruism – a quintessentially social phenotype (Sober and Wilson, 1998). To this end, some studies have already begun to probe the molecular underpinnings of altruistic traits such as alloparental care by workers (Amdam *et al.*, 2003a) and worker sterility (Thompson *et al.*, 2006), as well as the genetic basis of caste differentiation (Evans and Wheeler, 2000). Finding the genes that influence whether a genetically totipotent individual will develop into a member of the more- or less-reproductive caste, in response to pheromonal or nutritional cues (Winston, 1987), will help link the expression of social behaviour to developmental processes, and may thus reveal how developmental Bauplans (the generalized body plans of a group of animals) are modified by selection to promote social polyphenisms (Gadagkar, 1997; Evans and Wheeler, 2001). There remains also the possibility of identifying 'selfish genes' sensu Dawkins (1976), or their converse – 'genes for altruism'. In the honey bee, these might include genes that regulate worker sterility.

Consider a gene that has the effect of reducing the personal fitness of its bearer. If such a gene were expressed in all carriers, all the time, the gene would surely go extinct. Genes for altruism, which by definition reduce the direct fitness of its carrier, can therefore only persist if their expression in some individuals has the effect of increasing the reproductive fitness of others in which the gene is generally not expressed. This requirement for the conditional expression of genes for altruism can be exploited as a means to their isolation and identification, and molecular genomic techniques designed to capture genes via their conditional expression (e.g. subtractive hybridization, microarrays, differential display) could therefore prove a watershed for sociobiology.

For example, in normal wild-type honey bee colonies, the queen monopolizes reproduction to the exclusion of her daughters, the colony's workers. Workers are nonetheless capable of laying eggs parthenogenetically, but almost never do so in the presence of the queen and her brood, who signal their presence via pheromones (Visscher, 1989; Hoover *et al.*, 2003). The majority of workers can therefore be regarded as altruistic in the sociobiological sense, for they refrain from activating their ovaries and forgo their own reproductive opportunities in order to help the queen reproduce. By contrast, the very few (less than 1 in 10 000) workers that do activate their ovaries in queenright colonies can be considered selfish, for they attempt to exploit the reproductive capacity of their colony and rear their own sons. Candidate genes for altruism/selfishness might therefore simply be those that 'switch' worker ovaries *on* or *off* in queenright workers.

Interestingly, differences in worker propensity to activate their ovaries and lay eggs has a strong genetic basis. First, high rates of worker reproduction can be selected through controlled breeding (Oldroyd and Osborne, 1999; Barron *et al.*, 2001). Second, naturally occurring colonies where worker reproduction is present reveals that only one or a few patrilines are involved (Oldroyd *et al.*, 1994; Montague and Oldroyd, 1998; Châline *et al.*, 2002). Based on these observations, Oldroyd and Osborne (1999) suggested that variation in the regulation of worker reproduction is caused by differences at one, or at most a few, loci of major effect. While these genes have not yet been identified, we predict that studies of differential gene expression in ovary activated versus ovary deactivated workers will soon yield new candidates. A powerful approach will be to combine an open-ended gene expression screen (e.g. microarray) with a comprehensive QTL screen, the latter using markers from the near-saturated linkage maps available for the honey bee.

## 8 Conclusions

If an emphasis on molecular biology does accelerate the identification of genes that contribute to social variation, then a major step towards uniting sociobiological theory with molecular biology will have been taken, and a more comprehensive understanding of social life can then emerge. It is after all genes that are the kin-selected units that help shape social phenotypes over evolutionary time, and likewise that encode social phenotypes in real time. Characterizing these genes will therefore provide common ground for both functional and mechanistic biologists. Since core gene molecular function tends to be conserved across eukaryotes (Ashburner *et al.*, 2000), there is the expectation that genes that underlie the expression of social behaviours will also be conserved across taxa (Fitzpatrick *et al.*, 2005). We can then ask the question: 'Are there universal kin-selected genes

that promote social behaviour?' That is, are there certain genetic pre-conditions that, together with ecological ones (Crespi, 1994; Wilson and Hölldobler, 2005), promote the evolution of social behaviour? The answer to this question is not yet known and the molecular focus needed to answer it is only now beginning to be adopted (e.g. Amdam *et al.*, 2004). An explicitly molecular focus will therefore help us deduce the as-yet-unknown pathways that link variation in nucleotide sequence to variation in higher-order phenotypes that characterize social breeding systems. These pathways are assuredly convoluted, intersecting and easy to get lost in, but their deconstruction will serve as the unifying link that sociobiology needs, the predicted link between molecule and society (Bendall, 1983).

Behavioural genetic research in the age of genomics will not be without its challenges. Behavioural genetics has thus far been understood mostly in terms of population genetic models, which describe the evolution of social traits by parameterizing, abstractly, the genes that encode them. For example, it is not uncommon to read of 'genes for altruism' (Dawkins, 1976) or 'eusocial alleles' (Wilson and Hölldobler, 2005) in heuristic models of social evolution – unrealistic terms used in a figurative sense to convey abstract hypotheses in the absence of real gene identities. We hope that in the next few years, genes that control the major switches in caste determination and worker sterility will be identified.

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## References

Amdam, G. V., Norberg, K., Fondrk, M. K. and Page, R. E. (2004). Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. *Proc. Natl. Acad. Sci. USA* **101**, 11350–11355.

Amdam, G. V., Norberg, K., Hagen, A. and Omholt, S. W. (2003a). Social exploitation of vitellogenin. *Proc. Natl. Acad. Sci. USA* **100**, 1799–1802.

Amdam, G. V., Simoes, Z. L. P., Guidugli, K. R., Norberg, K. and Omholt, S. W. (2003b). Disruption of vitellogenin gene function in adult honeybees by intra-abdominal injection of double-stranded RNA. *BMC Biotechnol.* **3**, http://www.biomedcentral.com/1472-6750/1473/1471.

Anholt, R. R. H. and Mackay, T. F. C. (2004). Quantitative genetic analyses of complex behaviours in *Drosophila*. *Nat. Rev. Genet.* **5**, 838–849.

Arathi, H. S. and Spivak, M. (2001). Influence of colony genotypic composition on the performance of hygienic behaviour in the honeybee, *Apis mellifera* L. *Anim. Behav.* **62**, 57–66.

Arechavaleta-Velasco, M. E. and Hunt, G. J. (2003). Genotypic variation in the expression of guarding behavior and the role of guards in the defensive response of honey bee colonies. *Apidologie* **34**, 439–447.

Arechavaleta-Velasco, M. E. and Hunt, G. J. (2004). Binary trait loci that influence honey bee (Hymenoptera: Apidae) guarding behavior. *Ann. Entomol. Soc. Am.* **97**, 177–183.

Arechavaleta-Velasco, M. E., Hunt, G. J. and Emore, C. (2003). Quantitative trait loci that influence the expression of guarding and stinging behaviors of individual honey bees. *Behav. Genet.* **33**, 357–364.

Aronstein, K. and Saldivar, E. (2005). Characterization of a honey bee Toll related receptor gene Am18w and its potential involvement in antimicrobial immune defense. *Apidologie* **36**, 3–14.

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M. and Sherlock, G. (2000). Gene Ontology: tool for the unification of biology. *Nat. Genet.* **25**, 25–29.

Barron, A. B., Oldroyd, B. P. and Ratnieks, F. L. W. (2001). Worker reproduction in honey-bees (*Apis*) and the anarchic syndrome: a review. *Behav. Ecol. Sociobiol.* **50**, 199–208.

Barron, A. B., Schulz, D. J. and Robinson, G. E. (2002). Octopamine modulates responsiveness to foraging-related stimuli in honey bees (*Apis mellifera*). *J. Comp. Physiol. A.* **188**, 603–610.

Beekman, M., Doyen, L. and Oldroyd, B. P. (2005). Decrease in dance imprecision with increasing foraging distance in the honey bee *Apis mellifera* L. is partly explained by physical constraints. *J. Comp. Physiol. A.* **191**, 1107–1113.

Beling, I. (1929). Über des Zeitgedächtnis der Bienen. *Z. Vergl. Physiol.* **9**, 259–388.

Ben-Shahar, Y. (2005). The foraging gene, behavioral plasticity, and honeybee division of laboor. *J. Comp. Physiol. A.* **11**, 987–994.

Ben-Shahar, Y., Dudek, N. L. and Robinson, G. E. (2004). Phenotypic deconstruction reveals involvement of manganese transporter malvolio in honey bee division of labor. *J. Exp. Biol.* **207**, 3281–3288.

Ben-Shahar, Y., Leung, H. T., Pak, W. L., Sokolowski, M. B. and Robinson, G. E. (2003). cGMP-dependent changes in phototaxis: a possible role for the foraging gene in honey bee division of labor. *J. Exp. Biol.* **206**, 2507–2515.

Ben-Shahar, Y., Robichon, A., Sokolowski, M. B. and Robinson, G. E. (2002). Influence of gene action across different time scales on behavior. *Science* **296**, 741–744.

Bendall, D. S. (ed.) (1983). *Evolution from Molecules to Men*. Cambridge, MA: Cambridge University Press.

Beshers, S. N. and Fewell, J. H. (2001). Models of division of labor in social insects. *Ann. Rev. Entomol.* **46**, 413–440.

Beshers, S. N., Huang, Z. Y., Oono, Y. and Robinson, G. E. (2001). Social inhibition and the regulation of temporal polyethism in honey bees. *J. Theor. Biol.* **213**, 461–479.

Beutler, R. (1930). Biologisch-chemische Untersuchungen am Nektar von Immenblumen. *Z. Vergl. Physiol.* **12**, 72–176.

Beye, M., Hartel, S., Hagen, A., Hasselmann, M. and Omholt, S. W. (2002). Specific developmental gene silencing in the honey bee using a homeobox motif. *Ins. Mol. Biol.* **11**, 527–532.

Beye, M., Hasselmann, M., Fondrk, M. K., Page, R. E. and Omholt, S. W. (2003). The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein. *Cell* **114**, 419–429.

Beye, M., Poch, A., Burgtorf, C., Moritz, R. F. A. and Lehrach, H. (1998). A gridded genomic library of the honeybee (*Apis mellifera*): A reference library system for basic and comparative genetic studies of a hymenopteran genome. *Genomics* **49**, 317–320.

Bitterman, M. E. (1996). Comparative analysis of learning in honeybees. *Anim. Learn. Behav.* **24**, 123–141.

Bloch, G., Solomon, S. M., Robinson, G. E. and Fahrbach, S. E. (2003). Patterns of PERIOD and pigment-dispersing hormone immunoreactivity in the brain of the European honeybee (*Apis mellifera*): age- and time-related plasticity. *J. Comp. Neurol.* **464**, 269–284.

Bloch, G., Sullivan, J. P. and Robinson, G. E. (2002). Juvenile hormone and circadian locomotor activity in the honey bee *Apis mellifera*. *J. Insect Physiol.* **48**, 1123–1131.

Bloch, G., Toma, D. P. and Robinson, G. E. (2001). Behavioral rhythmicity, age, division of labor and period expression in the honey bee brain. *J. Biol. Rhythm* **16**, 444–456.

Boch, R. (1957). Rassenmäßige Unterschiede bei den Tänzen der Honigbiene (*Apis mellifica* L.). *Z. Vergl. Physiol.* **40**, 289–320.

Boch, R., Shearer, D. A. and Stone, B. C. (1962). Identification of iso-amyl acetate as an active component in the sting pheromone of the honeybees. *Nature* **195**, 1018–1020.

Bonabeau, E., Theraulaz, G. and Deneubourg, J. L. (1996). Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proc. R. Soc. Lond. B.* **263**, 1565–1569.

Bonabeau, E., Theraulaz, G. and Deneubourg, J. L. (1998). Fixed response thresholds and the regulation of division of labor in insect societies. *B. Math. Biol.* **60**, 753–807.

Bozic, J. and Woodring, J. (1998). Variations of brain biogenic amines in mature honeybees and induction of recruitment behavior. *Comp. Biochem. Phys. A.* **120**, 737–744.

Bozic, J. and Woodring, J. (2000). Variation in JH synthesis rate in mature honeybees and its possible role in reprogramming of hypopharyngeal gland function. *Pflugers Archiv-Euro. J. Physiol.* **439**, R163–R164.

Breed, M. D. (1991). Defensive behavior. In: *The "African" Honey Bee* (ed. Breed, M. D.), pp. 299–308. Boulder, CO: Westview Press.

Breed, M. D., Guzmán-Novoa, E. and Hunt, G. J. (2004). Defensive behavior of honey bees: organization, genetics, and comparisons with other bees. *Ann. Rev. Entomol.* **49**, 271–298.

Breed, M. D. and Rogers, K. B. (1991). The behavioral genetics of colony defense in honeybees: genetic variability for guarding behavior. *Behav. Genet.* **21**, 295–303.

Breed, M. D., Rogers, K. B., Hunley, J. A. and Moore, A. J. (1989). A correlation between guard behavior and defensive response in the honey bee, *Apis mellifera*. *Anim. Behav.* **37**, 515–516.

Breed, M. D., Smith, T. A. and Torres, A. (1992). Role of guard honey bees (Hymenoptera, Apidae) in nestmate discrimination and replacement of removed guards. *Ann. Entomol. Soc. Am.* **85**, 633–637.

Butler, C. G. and Free, J. B. (1952). The behaviour of worker honey bees at the hive entrance. *Behaviour* **4**, 262–292.

Calderone, N. W. (1998). Proximate mechanisms of age polyethism in the honey bee, *Apis mellifera* L. *Apidologie* **29**, 127–158.

Calderone, N. W. and Page, R. E. (1988). Genotypic variability in age polyethism and task specialization in the honey bee, *Apis mellifera* (Hymenoptera: Apidae). *Behav. Ecol. Sociobiol.* **22**, 17–25.

Calderone, N. W. and Page, R. E. (1991). Evolutionary genetics of division of labor in colonies of the honey bee (*Apis mellifera*). *Am. Nat.* **138**, 69–92.
Calderone, N. W. and Page, R. E. (1992). Effects of interactions among genotypically diverse nestmates on task specialization by foraging honey bees (*Apis mellifera*). *Behav. Ecol. Sociobiol.* **30**, 219–226.
Calderone, N. W. and Page, R. E. (1996). Temporal polyethism and behavioural canalization in the honey bee, *Apis mellifera*. *Anim. Behav.* **51**, 631–643.
Calderone, N. W., Robinson, G. E. and Page, R. E. (1989). Genetic structure and division of labor in honeybee societies. *Experientia* **45**, 765–767.
Camp, N. J. and Cox, M. D. (eds) (2002). *Quantitative Trait Loci: Methods and Protocols*. Totowa, NJ: Humana Press.
Châline, N., Ratnieks, F. L. W. and Bourke, T. (2002). Anarchy in the UK: detailed genetic analysis of worker reproduction in a naturally-occurring British anarchistic honeybee, *Apis mellifera*, colony using DNA microsatellites. *Mol. Ecol.* **11**, 1795–1803.
Châline, N., Ratnieks, F. L. W., Raine, N. E., Badcock, N. S. and Burke, T. (2004). Non-lethal sampling of honey bee, *Apis mellifera,* DNA using wing tips. *Apidologie* **35**, 311–318.
Chandra, S. B. C., Hosler, J. S. and Smith, B. H. (2000). Heritable variation for latent inhibition and its correlation with reversal learning in honeybees (*Apis mellifera*). *J. Comp. Psychol.* **114**, 86–97.
Chandra, S. B. C., Hunt, G. J., Cobey, S. and Smith, B. H. (2001). Quantitative trait loci associated with reversal learning and latent inhibition in honeybees (*Apis mellifera*). *Behav. Genet.* **31**, 275–285.
Collins, A. M., Brown, M. A., Rinderer, T. E., Harbo, J. R. and Tucker, K. W. (1987a). Heritabilities of honeybee alarm pheromone production. *J. Hered.* **78**, 29–31.
Collins, A. M. and Kubasek, K. J. (1982). Field test of honey bee (Hymenoptera: Apidae) defensive behavior. *Ann. Entomol. Soc. Am.* **75**, 383–387.
Collins, A. M., Rinderer, T. E., Harbo, J. R. and Bolten, A. B. (1982). Colony defense by Africanized and European bees. *Science* **218**, 72–74.
Collins, A. M., Rinderer, T. E., Harbo, J. R. and Brown, M. A. (1984). Heritabilities and genetic correlations for several characters in the honey bee. *J. Hered.* **75**, 135–140.
Collins, A. M., Rinderer, T. E., Tucker, K. W. and Pesante, D. G. (1987b). Response to alarm pheromone by European and Africanized honeybees. *J. Apic. Res.* **26**, 217–223.
Collins, A. M., Rinderer, T. E., Tucker, K. W., Sylvester, H. A. and Lackett, J. J. (1980). A model of honeybee defensive behaviour. *J. Apic. Res.* **19**, 224–231.
Crailsheim, K., Hrassnigg, N. and Stabentheiner, A. (1996). Diurnal behavioural differences in forager and nurse honey bees (*Apis mellifera carnica* Pullm). *Apidologie* **27**, 235–244.
Crespi, B. J. (1994). Three conditions for the evolution of eusociality – Are they sufficient? *Insectes Soc.* **41**, 395–400.
Dawkins, R. (1976). *The Selfish Gene*. Oxford, UK: Oxford University Press.
Debelle, J. S. and Sokolowski, M. B. (1987). Heredity of rover/sitter: alternative foraging strategies of *Drosophila melanogaster* larvae. *Heredity* **59**, 73–83.
deGrandi-Hoffman, G., Collins, A., Martin, J. H., Schmidt, J. O. and Spangler, H. G. (1998). Nest defence behavior in colonies from crosses between Africanized and European honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae). *J. Insect Behav.* **11**, 37–45.

Doerge, R. W. (2002). Mapping and analysis of quantitative trait loci in experimental populations. *Nat. Rev. Genet.* **3**, 43–52.
Downs, S. G. and Ratnieks, F. L. W. (1999). Recognition of conspecifics by honeybee guards uses nonheritable cues acquired in the adult stage. *Anim. Behav.* **58**, 643–648.
Dreller, C. (1998). Division of labor between scouts and recruits – genetic influence and mechanisms. *Behav. Ecol. Sociobiol.* **43**, 191–196.
Dreller, C., Page, R. E. and Fondrk, M. K. (1999). Regulation of pollen foraging in honeybee colonies: effects of young brood, stored pollen, and empty space. *Behav. Ecol. Sociobiol.* **45**, 227–233.
Dreller, C. and Tarpy, D. R. (2000). Perception of the pollen need by foragers in a honeybee colony. *Anim. Behav.* **59**, 91–96.
Dyer, F. C. and Seeley, T. D. (1991). Dance dialects and foraging range in three Asian honey bee species. *Behav. Ecol. Sociobiol.* **28**, 227–233.
Ebbersten, K. (1996). Genetic relationships, generation interval and effective population size in the honey bee (*Apis mellifera* L.). Ph.D. thesis. Department of Animal Breeding and Genetics, Swedish University of Agricultural Scences, Uppsala, Sweden.
Eckert, C. D., Winston, M. L. and Ydenberg, R. C. (1994). The relationship between population size, amont of brood, and individual foraging behaviour in the honey bee, *Apis mellifera* L. *Oecologia* **97**, 248–255.
Esch, H. E., Zhang, S., Srinivasen, M. V. and Tautz, J. (2001). Honeybee dances communicate distances by optic flow. *Nature* **411**, 581–583.
Evans, J. D., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J.-L., Jiang, H., Kanost, M., Thompson, G. J., Zou, Z. and Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees *Apis mellifera. Ins. Mol. Biol.* **15**, 645–656.
Evans, J. D. and Wheeler, D. E. (2001). Expression profiles during honeybee caste determination. *Genome Biol.* http://genomebiology.com/content/2/1/research0001.1
Evans, J. D. and Wheeler, D. E. (2001). Gene expression and the evolution of insect polyphenisms. *Bioessays* **23**, 62–68.
Fahrbach, S. E. and Robinson, G. E. (1996). Juvenile hormone, behavioral maturation, and brain structure in the honey bee. *Dev. Neurosci.* **18**, 102–114.
Falconer, D. S. and Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*. 4th edition Essex, UK: Prentice-Hall.
Farooqui, T., Robinson, K., Vaessin, H. and Smith, B. H. (2003). Modulation of early olfactory processing by an octopaminergic reinforcement pathway in the honeybee. *J. Neurosci.* **23**, 5370–5380.
Farooqui, T., Vaessin, H. and Smith, B. H. (2004). Octopamine receptors in the honeybee (*Apis mellifera*) brain and their disruption by RNA-mediated interference. *J. Insect Physiol.* **50**, 701–713.
Ferguson, H. J., Cobey, S. and Smith, B. H. (2001). Sensitivity to a change in reward is heritable in the honeybee, *Apis mellifera*. *Anim. Behav.* **61**, 527–534.
Feuerbacher, E., Fewell, J. H., Roberts, S. P., Smith, E. F. and Harrison, J. F. (2003). Effects of load type (pollen or nectar) and load mass on hovering metabolic rate and mechanical power output in the honey bee *Apis mellifera*. *J. Exp. Biol.* **206**, 1855–1865.
Fewell, J. H. (2003). Social insect networks. *Science* **301**, 1867–1870.
Fewell, J. H. and Bertram, S. M. (1999). Division of labor in a dynamic environment: response by honeybees (*Apis mellifera*) to graded changes in colony pollen stores. *Behav. Ecol. Sociobiol.* **46**, 171–179.

Fewell, J. H. and Page, R. E. (1993). Genotypic variation in foraging responses to environmental stimuli by honey bees, *Apis mellifera*. *Experientia* **49**, 1106–1112.
Fewell, J. H. and Page, R. E. (2000). Colony-level selection effects on individual and colony foraging task performance in honeybees, *Apis mellifera* L. *Behav. Ecol. Sociobiol.* **48**, 173–181.
Fewell, J. H. and Winston, M. L. (1992). Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. *Behav. Ecol. Sociobiol.* **30**, 387–393.
Fitzpatrick, M. J., Ben-Shahar, Y., Smid, H. M., Vet, L. E. M., Robinson, G. E. and Sokolowski, M. B. (2005). Candidate genes for behavioural ecology. *Trends Ecol. Evol.* **20**, 96–104.
Frisch, B. and Ascoff, J. (1987). Circadian rhythms in honeybees: entrainment by feeding cycles. *Physiol. Entomol.* **12**, 41–49.
Frumhoff, P. C. and Baker, J. (1988). A genetic component to division of labour within honey bee colonies. *Nature* **333**, 358–361.
Fuchs, S. and Moritz, R. F. A. (1998). Evolution of extreme polyandry in the honeybee *Apis mellifera* L. *Behav. Ecol. Sociobiol.* **39**, 269–275.
Gadagkar, R. (1997). The evolution of caste polymorphism in social insects: genetic release followed by diversifying evolution. *J. Genet.* **76**, 167–179.
Ghent, R. L. and Gary, N. E. (1962). A chemical releaser in honey bee stings (*Apis mellifera* L.). *Psyche* **69**, 1–6.
Giray, T., Guzmán-Novoa, E., Aron, C. W., Zelinsky, B., Fahrbach, S. E. and Robinson, G. E. (2000). Genetic variation in worker temporal polyethism and colony defensiveness in the honey bee, *Apis mellifera*. *Behav. Ecol.* **11**, 44–55.
Giray, T., Huang, Z.-Y., Guzmán-Novoa, E. and Robinson, G. E. (1999). Physiological correlates of genetic variation for rate of behavioral development in the honeybee, *Apis mellifera*. *Behav. Ecol. Sociobiol.* **47**, 17–28.
Giray, T. and Robinson, G. E. (1994). Effects of intracolony variability in behavioral development on plasticity of division of labor in honey bee colonies. *Behav. Ecol. Sociobiol.* **35**, 13–20.
Giray, T. and Robinson, G. E. (1996). Common endocrine and genetic mechanisms of behavioral development in male and worker honey bees and the evolution of division of labor. *Proc. Natl. Acad. Sci. USA* **93**, 11718–11722.
Graham, S., Myerscough, M. R., Jones, J. and Oldroyd, B. P. (2006). Modelling the role of intracolonial genetic diversity on regulation of brood temperature in honey bee (*Apis mellifera* L.) colonies. *Insectes Soc.* **52** in press.
Gregory, P. G. and Rinderer, T. E. (2004). Non-destructive sources of DNA used to genotype honey bee (*Apis mellifera*) queens. *Ent. Exp. Appl.* **111**, 173–177.
Grozinger, C. M., Sharabash, N. M., Whitfield, C. W. and Robinson, G. E. (2003). Pheromone-mediated gene expression in the honey bee brain. *Proc. Natl. Acad. Sci. USA* **100**, 14519–14525.
Guidugli, K. R., Nascimento, A. M., Amdam, G. V., Barchuk, A. R., Omholt, S., Simoes, Z. L. P. and Hartfelder, K. (2005). Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. *FEBS Lett.* **579**, 4961–4965.
Guzmán-Novoa, E., Hunt, G. J., Uribe, J. L., Smith, C. and Arechavaleta-Velasco, M. E. (2002). Confirmation of QTL effects and evidence of genetic dominance of honeybee defensive behavior: results of colony and individual behavioral assays. *Behav. Genet.* **32**, 95–102.
Guzmán-Novoa, E. and Page, R. E. (1993). Backcrossing Africanized honey bee queens to European drones reduces colony defensive behavior, Ann. *Entomol. Soc. Am.* **86**, 352–355.
Guzmán-Novoa, E. and Page, R. E. (1994). Genetic dominance and worker interactions affect honeybee colony defense. *Behav. Ecol.* **5**, 91–97.

Guzmán-Novoa, E., Page, R. E. and Gary, N. E. (1994). Behavioral and life-history components of division of labor in honey bees (*Apis mellifera*). *Behav. Ecol. Sociobiol.* **34**, 409–417.

Guzmán-Novoa, E., Page, R. E. J., Spangler, H. G. and Erickson, E. H. (1999). A comparison of two assays to test the defensive behaviour of honey bees (*Apis mellifera*). *J. Apicult. Res.* **38**, 205–209.

Hamilton, W.D. (1996). Narrow roads of gene land. The Collected Papers of W. D. Hamilton. *Evolution of Social Behaviour*: Vol. I. Oxford, UK: W. H. Freeman.

Hammer, M. and Menzel, R. (1998). Multiple sites of associative odor learning as revealed by local brain microinjections of octopamine in honeybees. *Learn. Memory* **5**, 146–156.

Harbo, J. R. (1986). Propagation and instrumental insemination. In: *Bee Genetics and Breeding* (ed. Rinderer, T. E.), pp. 361–389. Orlando, FL: Academic Press.

Harbo, J. R. (1990). Artificial mixing of spermatozoa from honey bees and evidence for sperm competition. *J. Apic. Res.* **29**, 151–158.

Hasselmann, M., Fondrk, M. K., Page, R. E. and Beye, M. (2001). Fine scale mapping in the sex locus region of the honey bee (*Apis mellifera*). *Ins. Mol. Biol.* **10**, 605–608.

Hellmich, R. L., Kulincevic, J. M. and Rothenbuhler, W. C. (1985). Selection for high and low pollen-hoarding honey bees. *J. Hered.* **76**, 155–158.

Hillesheim, E., Koeniger, N. and Moritz, R. F. A. (1989). Colony performance in honeybees (*Apis mellifera capensis* Esch.) depends on the proportion of subordinate and dominant workers. *Behav. Ecol. Sociobiol.* **24**, 291–296.

Honey Bee Genome Sequencing Consortium. (2006). Insights into social insects from the genome of the honey bee *Apis mellifera. Nature* **443**, 931–949.

Hoover, S. E. R., Keeling, C. I., Winston, M. L. and Slessor, K. N. (2003). The effect of queen pheromones on worker honey bee ovary development. *Naturwissenschaften* **90**, 477–480.

Huang, Z. Y., Robinson, G. E. and Borst, D. W. (1994). Physiological correlates of division of labor among similarly aged honey bees. *J. Comp. Physiol. A* **174**, 731–739.

Huang, Z. Y. and Robinson, G. E. (1992). Honeybee colony integration: worker-worker interactions mediate hormonally regulated plasticity in division of labor. *Proc. Natl. Acad. Sci. USA* **89**, 11726–11729.

Huang, Z. Y. and Robinson, G. E. (1996). Regulation of honey bee division of labor by colony age demography. *Behav. Ecol. Sociobiol.* **39**, 147–158.

Huang, Z. Y. and Robinson, G. E. (1999). Social control of division of labor in honey bee colonies. In: *Information Processing in Social Insects* (eds Detrain, C. Deneubourg, J. L. and Pasteels, J. M.), pp. 165–186. Basel, Switzerland: Birkhäuser.

Humphries, M. A., Muller, U., Fondrk, M. K. and Page, R. E. (2003). PKA and PKC content in the honey bee central brain differs in genotypic strains with distinct foraging behavior. *J. Comp. Physiol. A* **189**, 555–562.

Hunt, G. J., Collins, A. M., Rivera, R., Page, R. E. and Guzman-Novoa, E. (1999). Quantitative trait loci influencing honeybee alarm pheromone levels. *J. Hered.* **90**, 585–589.

Hunt, G. J., Guzman-Novoa, E., Fondrk, M. K. and Page, R. E. (1998). Quantitative trait loci for honey bee stinging behavior and body size. *Genetics* **148**, 1203–1213.

Hunt, G. J., Guzmán-Novoa, E., Uribe-Rubio, J. L. and Prieto-Merlos, D. (2003). Genotype–environment interactions in honeybee guarding behaviour. *Anim. Behav.* **66**, 459–467.

Hunt, G. J. and Page, R. E. (1995). Linkage map of the honey bee, *Apis mellifera*, based on RAPD markers. *Genetics* **139**, 1371–1382.

Hunt, G. J., Page, R. E., Fondrk, M. K. and Dullum, C. J. (1995). Major quantitative trait loci affecting honey bee foraging behavior. *Genetics* **141**, 1537–1545.

Iwasaki, K. and Thomas, J. H. (1997). Genetics in rhythm. *Trends Gen.* **13**, 111–115.

Jackson, R. B., Linder, C. R., Lynch, M., Purugganan, M., Somerville, S. and Thayer, S. S. (2002). Linking molecular insight and ecological research. *Trends Ecol. Evol.* **17**, 409–414.

Janmaat, A. F. and Winston, M. L. (2000). Condition-dependent response to changes in pollen stores by honey bee (*Apis mellifera*) colonies with different parasitic loads. *Behav. Ecol. Sociobiol.* **47**, 171–179.

Jansen, R. C. and Nap, J. P. (2001). Genetical genomics: the added value from segregation. *Trends Genet.* **17**, 388–391.

Jassim, O., Huang, Z. Y. and Robinson, G. E. (2000). Juvenile hormone profiles of worker honey bees, *Apis mellifera*, during normal and accelerated behavioural development. *J. Insect Physiol.* **46**, 243–249.

Johnson, R. N., Oldroyd, B. P., Barron, A. B. and Crozier, R. H. (2002). Genetic control of the honey bee (*Apis mellifera*) dance language: Segregating dance forms in a backcrossed colony. *J. Hered.* **93**, 170–173.

Jones, J., Myerscough, M., Graham, S. and Oldroyd, B. P. (2004). Honey bee nest thermoregulation: diversity promotes stability. *Science* **305**, 402–404.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.

Koeniger, N. and Koeniger, G. (1991). An evolutionary approach to mating behaviour and drone copulatory organs in *Apis*. *Apidologie* **22**, 581–590.

Kolmes, S. A., Winston, M. L. and Furgusson, L. A. (1989). The division of labor among worker honey bees (Hymenoptera: Apidae): the effects of multiple patrilines. *J. Kans. Ent. Soc.* **62**, 80–95.

Konopka, R. J., Kyriacou, C. P. and Hall, J. C. (1996). Mosaic analysis in the Drosophila CNS of circadian and courtship-song rhythms affected by a period clock mutation. *J. Neurogenet* **11**, 117–140.

Kryger, P., Kryger, U. and Moritz, R. F. A. (2000). Genotypical variability for the tasks of water collecting and scenting in a honey bee colony. *Ethology* **106**, 769–779.

Kuwabara, M. (1957). Bildung des bedingten Reflexes von Pavlovs Tyous bei der Honigbene, *Apis mellifera*. *J. Faculty Sci. Hokkaido Univ. S. VI Zool.* **13**, 458–464.

Laidlaw, H. H. and Page, R. E. (1984). Polyandry in honey bees (*Apis mellifera* L.): sperm utilization and intracolony genetic relationships. *Genetics* **108**, 985–997.

Laidlaw, H. H. and Page, R. E. J. (1997). *Queen Rearing and Bee Breeding*. Cheshire, USA: Wicwas Press.

Lander, E. S. and Botstein, D. (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185–199.

Lapidge, K. L., Oldroyd, B. P. and Spivak, M. (2002). Seven suggestive quantitative trait loci influence hygienic bahavior of honey bees. *Naturwissenschaften* **89**, 565–568.

Leoncini, I., Le Conte, Y., Costagliola, G., Plettner, E., Toth, A. L., Wang, M. W., Huang, Z., Becard, J. M., Crauser, D., Slessor, K. N. and Robinson, G. E. (2004). Regulation of behavioral maturation by a primer pheromone produced by adult worker honey bees. *P. Natl. Acad. Sci. USA* **101**, 17559–17564.

Lindauer, M. (1956). Über die Verstänigung bie indischen Bienen. *Z. Vergl. Physiol.* **38**, 521–527.
Lindauer, M. (1957). Communication among the honeybees and stingless bees of India. *Bee World,* **38**, 3–14, 34–39.
Lindauer, M. (1960). Time-compensated sun orientation in bees. *Cold Spring. Harb. Sym.* **25**, 371–377.
Lindauer, M. (1971). *Communication Among Social Bees*. Cambridge, MA: Harvard University Press.
Liu, B.-H. (1998). *Statistical Genomics: Linkage, Mapping, and QTL Analysis*. Boca Raton, FL: CRC Press.
Lobo, N. F., Ton, L. Q., Hill, C. A., Emore, C., Romero-Severson, J., Hunt, G. J. and Collins, F. H. (2003). Genomic analysis in the sting-2 quantitative trait locus for defensive behavior in the honey bee, *Apis mellifera*. *Genome Res.* **13**, 2588–2593.
Martin, C. J., Oldroyd, B. P. and Beekman, M. (2004). Differential reproductive success among subfamilies in queenless honey bee colonies (*Apis mellifera* L.). *Behav. Ecol. Sociobiol.* **56**, 42–49.
Masterman, R., Ross, R., Mesce, K. and Spivak, M. (2001). Olfactory and behavioral response thresholds to odors of diseased brood differ between hygienic and non-hygienic honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A.* **187**, 441–452.
Masterman, R., Smith, B. H. and Spivak, M. (2000). Brood odor discrimination abilities in hygienic honey bees (*Apis mellifera* L.) using proboscis extension reflex conditioning. *J. Insect Behav.* **13**, 87–101.
Menzel, R. (1999). Memory dynamics in the honeybee. *J. Comp. Physiol. A.* **185**, 323–340.
Menzel, R. and Müller, U. (1996). Learning and memory in honeybees: from behavior to neural substrates. *Annu. Rev. Neurosci.* **19**, 379–404.
Montague, C. E. and Oldroyd, B. P. (1998). The evolution of worker sterility in honey bees: an investigation into a behavioral mutant causing failure of worker policing. *Evolution* **52**, 1408–1415.
Moore, A. J., Breed, M. D. and Moor, M. J. (1987). The guard honey bee; ontogeny and behavioral variability of workers performing a specialized task. *Anim. Behav.* **35**, 1159–1167.
Moore, D., Angel, J. F., Cheeseman, I. M., Fahrbach, S. E. and Robinson, G. E. (1998). Timekeeping in the honey bee colony: integration of circadian rhythms and division of labor. *Behav. Ecol. Sociobiol.* **43**, 147–160.
Moore, D. and Rankin, M. A. (1985). Circadian locomotor rhythms in individual honeybees. *Physiol. Entomol.* **10**, 191–197.
Moritz, R. F. A. (1988). A reevaluation of the two-locus model for hygienic behavior in honeybees (*Apis mellifera* L.). *J. Hered.* **79**, 257–262.
Moritz, R. F. A. and Southwick, E. E. (1987). Phenotypic interactions in group behavior of honey bee workers (*Apis mellifera* L.). *Behav. Ecol. Sociobiol.* **21** .
Myerscough, M. and Oldroyd, B. P. (2004). Simulation models of the role of genetic variability in social insect task allocation. *Insectes Soc.* **51**, 146–152.
O'Donnell, S. and Foster, R. L. (2001). Thresholds of response in nest thermoregulation by worker bumble bees, *Bombus bifarius nearcticus* (Hymenoptera: Apidae). *Ethology* **107**, 387–399.
Oldroyd, B. P. and Osborne, K. E. (1999). The evolution of worker sterility in honeybees: the genetic basis of failure of worker policing. *Proc. R. Soc. Lond. B.* **266**, 1335–1339.
Oldroyd, B. P., Rinderer, T. E. and Buco, S. M. (1991a). Honey bees dance with their super-sisters. *Anim. Behav.* **42**, 121–129.

Oldroyd, B. P., Rinderer, T. E. and Buco, S. M. (1991b). Intracolonial variance in honey bee foraging behaviour: the effects of sucrose concentration. *J. Apic. Res.* **30**, 137–145.

Oldroyd, B. P., Rinderer, T. E. and Buco, S. M. (1992). Intra-colonial foraging specialism by honey bees (*Apis mellifera*) (Hymenoptera: Apidae). *Behav. Ecol. Sociobiol.* **30**, 292–295.

Oldroyd, B. P., Rinderer, T. E., Buco, S. M. and Beaman, L. D. (1993). Genetic variance in honey bees for preferred foraging distance. *Anim. Behav.* **45**, 323–332.

Oldroyd, B. P., Smolenski, A. J., Cornuet, J. M. and Crozier, R. H. (1994). Anarchy in the beehive. *Nature* **371**, 749.

Oldroyd, B. P. and Wongsiri, S. (2006). *Asian Honey Bees. Biology, Conservation and Human Interactions*. Cambridge, MA: Harvard University Press.

Osborne, K. A., Robichon, A., Burgess, E., Butland, S., Shaw, R. A., Coulthard, A., Pereira, H. S., Greenspan, R. J. and Sokolowski, M. B. (1997). Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila*. *Science* **277**, 834–836.

Page, R. E. and Erber, J. (2002). Levels of behavioral organization and the evolution of division of labor. *Naturwissenschaften* **89**, 91–106.

Page, R. E., Erber, J. and Fondrk, M. K. (1998). The effect of genotype on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A.* **182**, 489–500.

Page, R. E. and Fondrk, M. K. (1995). The effects of colony-level selection on the social organization of honey bee (*Apis mellifera* L.) colonies: colony-level components of pollen hoarding. *Behav. Ecol. Sociobiol.* **36**, 135–144.

Page, R. E., Fondrk, M. K., Hunt, G. J., Guzmán-Novoa, E., Humphries, M. A., Nguyen, K. and Greene, A. S. (2000). Genetic dissection of honeybee (*Apis mellifera* L.) foraging behavior. *J. Hered.* **91**, 474–479.

Page, R. E., Gadau, J. and Beye, M. (2002). The emergence of hymenopteran genetics. *Genetics* **160**, 375–379.

Page, R. E. and Laidlaw, H. H. (1982). Closed population honeybee breeding. 1. Population genetics of sex determination. *J. Apic. Res.* **21**, 30–37.

Page, R. E. and Laidlaw, H. H. (1988). Full sisters and super sisters: a terminological paradigm. *Anim. Behav.* **36**, 944–945.

Page, R. E. and Mitchell, S. D. (1998). Self-organization and the evolution of division of labor. *Apidologie* **29**, 171–190.

Page, R. E. and Peng, C. Y. S. (2001). Aging and development in social insects with emphasis on the honey bee, *Apis mellifera* L. *Exp. Gerontol.* **36**, 695–711.

Page, R. E., Robinson, G. E., Britton, D. S. and Fondrk, M. K. (1991). Genotypic variability for rates of behavioral development in worker honeybees (*Apis mellifera* L.). *Behav. Ecol.* **3**, 173–180.

Page, R. E., Robinson, G. E., Calderone, N. W. and Rothenbuhler, W. C. (1989a). Genetic structure, division of labor, and the evolution of insect societies. In: *The Genetics of Social Evolution* (eds Breed, M. D. and Page, R. E.), pp. 15–29. Boulder, CO: Westview Press.

Page, R. E., Robinson, G. E. and Fondrk, M. K. (1989b). Genetic specialists, kin recognition and nepotism in honey-bee colonies. *Nature* **338**, 576–579.

Page, R. E., Waddington, K. D., Hunt, G. J. and Fondrk, M. K. (1995). Genetic determinants of honey bee foraging behavior. *Anim. Behav.* **50**, 1617–1625.

Palmer, K. A. and Oldroyd, B. P. (2000). Evolution of multiple mating in the genus *Apis*. *Apidologie* **31**, 235–248.

Pankiw, T., Nelson, M., Page, R. E. and Fondrk, M. K. (2004). The communal crop: modulation of sucrose response thresholds of pre-foraging honey bees with incoming nectar quality. *Behav. Ecol. Sociobiol.* **55**, 286–292.
Pankiw, T. and Page, R. E. (1999). The effect of genotype, age, sex, and caste on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A.* **185**, 207–213.
Pankiw, T. and Page, R. E. (2000). Response thresholds to sucrose predict foraging division of labor in honeybees. *Behav. Ecol. Sociobiol.* **47**, 265–267.
Pankiw, T. and Page, R. E. (2001). Brood pheromone modulates honeybee (*Apis mellifera* L.) sucrose response thresholds. *Behav. Ecol. Sociobiol.* **49**, 206–213.
Pankiw, T. and Page, R. E. (2003). Effect of pheromones, hormones, and handling on sucrose response thresholds of honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A.* **189**, 675–684.
Pankiw, T., page, R. E. and Fondrk, M. K. (1998). Brood pheromone stimulates pollen foraging in honey bees (Apis mellifera). *Behave. Ecol. Sociobiol.* **44**, 193–198.
Pankiw, T., Tarpy, D. R. and Page, R. E. J. (2002). Genotype and rearing environment affect honeybee perception and foraging environment. *Anim. Behav.* **64**, 663–672.
Pereira, H. S. and Sokolowski, M. B. (1993). Mutations in the larval foraging gene affect adult locomotory behavior after feeding in *Drosophila melanogaster*. *P. Natl. Acad. Sci. USA* **90**, 5044–5046.
Price, J. L., Dembinska, M. E., Young, M. W. and Rosbash, M. (1995). Suppression of PERIOD protein abundance and circadian cycling by the *Drosphila* clock mutation timeless. *EMBO J.* **14**, 4044–4099.
Punchihewa, R. K. W., Koeniger, N., Kevan, P. G. and Gadawaski, R. M. (1985). Observations on the dance communication and natural foraging ranges of *Apis cerana*, *Apis dorsata*, and *Apis florea* in Sri Lanka. *J. Apic. Res.* **24**, 168–175.
Queller, D. C. and Strassmann, J. E. (1998). Kin selection and social insects. *Bioscience* **48**, 165–175.
Rani, U. and Jain, K. L. (1997). Age related differences in taste responses to sugars in *Apis* species. *Indian J. Exp. Biol.* **35**, 470–472.
Reeve, H. K. and Keller, L. (1999). Levels of selection: burying the units-of-selection debate and unearthing the crucial new issues. In: *Levels of Selection in Evolution* (ed. Keller, L.), pp. 3–14. Princeton, NJ: Princeton University Press.
Remington, D. L., Ungerer, M. C. and Purugganan, M. D. (2001). Map-based cloning of quantitative trait loci: progress and prospects. *Genet. Res.* **78**, 213–218.
Renner, M. (1955). Über die Haltung von Bienen in geschlossenen, künstlich beleuchteten Råumen. *Naturwissenschaften* **42**, 539–540.
Renner, M. (1957). Neue Versuche Über den Zietsinn der Honigbiene. *Z. Vergl. Physiol.* **40**, 85–118.
Ribbands, C. R. (1954). The defence of the honeybee community. *Proc. Roy. Soc. B* **142**, 514–524.
Rinderer, T. E. (ed.) (1986). *Bee Genetics and Breeding*. Orlando, FL: Academic Press.
Rinderer, T. E. and Beaman, L. D. (1995). Genic control of honey bee dance language dialect. *Theor. Appl. Genet.* **91**, 727–732.
Rinderer, T. E., Oldroyd, B. P., Wongsiri, S., Sylvester, H. A., de, G. L., Potichot, S., Sheppard, W. S. and Buchmann, S. L. (1993). Time of drone flight in four honey bee species in south-eastern Thailand. *J. Apicult. Res.* **32**, 27–33.
Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* **37**, 637–665.

Robinson, G. E. and Ben-Shahar, Y. (2002). Social behavior and comparative genomics: new genes or new gene regulation? *Genes Brain Behav.* **1**, 197–203.
Robinson, G. E., Fahrbach, S. E. and Winston, M. L. (1997). Insect societies and the molecular biology of social behavior. *Bioessays* **19**, 1099–1108.
Robinson, G. E. and Huang, Z. Y. (1998). Colony integration in honey bees: genetic, endocrine and social control of division of labor. *Apidologie* **29**, 159–170.
Robinson, G. E. and Page, R. E. (1988). Genetic determination of guarding and undertaking in honey bee colonies. *Nature* **333**, 356–358.
Robinson, G. E. and Page, R. E. (1989a). Genetic basis for division of labor in an insect society. In: *The Genetics of Social Evolution* (eds Breed, M. D. and Page, R. E.), pp. 61–80. Boulder, CO: Westview Press.
Robinson, G. E. and Page, R. E. (1989b). Genetic determination of nectar foraging, pollen foraging and nest-site scouting in honey bee colonies. *Behav. Ecol. Sociobiol.* **24**, 317–323.
Robinson, G. E., Page, R. E. and Arensen, N. (1994). Genotypic differences in brood rearing in honey bee colonies: context specific? *Behav. Ecol. Sociobiol.* **34**, 125–137.
Robinson, G. E., Page, R. E. and Fondrk, M. K. (1990). Intracolonial behavioral variation in worker oviposition, oophagy, and larval care in queenless honey bee colonies. *Behav. Ecol. Sociobiol.* **26**, 315–323.
Robinson, G. E., Page, R. E., Strambi, C. and Strambi, A. (1989). Hormonal and genetic control of behavioral integration in honey bee colonies. *Science* **246**, 109–111.
Robinson, G. E., Strambi, C., Strambi, A. and Huang, Z.-Y. (1992). Reproduction of worker honey bees is associated with low juvenile hormone titres and rates of biosynthesis. *Gen. Comp. Endocrinol.* **87**, 471–480.
Rothenbuhler, W. C. (1964). Behavior genetics of nest cleaning in honey bees. IV. Responses of F1 and backcross generations to disease-killed brood. *Am. Zool.* **12**, 578–583.
Rothenbuhler, W. C. and Page, R. E. (1989). Genetic variability for temporal polyethism in colonies consisting of similarly-aged worker honey bees. *Apidologie* **20**, 433–437.
Rotjan, R. D., Calderone, N. W. and Seeley, T. D. (2002). How a honey bee colony mustered additional labor for the task of pollen foraging. *Apidologie* **33**, 367–373.
Rueppell, O., Chandra, S. B. C., Pankiw, T., Fondrk, M. K., Beye, M., Hunt, G. and Page, R. E. (2006). The genetic architecture of sucrose responsiveness in the honeybee (*Apis mellifera* L.). *Genetics* **172**, 243–251.
Rueppell, O., Pankiw, T., Nielsen, D. I., Fondrk, M. K., Beye, M. and Page, R. E. (2004a). The genetic architecture of the behavioral ontogeny of foraging in honeybee workers. *Genetics* **167**, 1767–1779.
Rueppell, O., Pankiw, T. and Page, R. E. (2004b). Pleiotropy, epistasis and new QTL: The genetic architecture of honey bee foraging behavior. *J. Hered.* **95**, 481–491.
Ruttner, F. (1988). *Biogeography and Taxonomy of Honeybees*. Berlin: Springer.
Scheiner, R., Barnert, M. and Erber, J. (2003a). Variation in water and sucrose responsiveness during the foraging season affects proboscis extension learning in honey bees. *Apidologie* **34**, 67–72.
Scheiner, R., Müller, U., Heimburger, S. and Erber, J. (2003b). Activity of protein kinase A and gustulatory responsiveness in the honey bee (*Apis mellifera* L.). *J. Comp. Physiol. A* **189**, 427–434.

Scheiner, R., Page, R. E. and Erber, J. (2001). The effects of genotype, foraging role, and sucrose responsiveness on the tactile learning performance of honey bees (*Apis mellifera* L.). *Neurobiol. Learn. Mem.* **76**, 138–150.
Scheiner, R., Page, R. E. and Erber, J. (2004). Sucrose responsiveness and behavioral plasticity in honey bees (*Apis mellifera*). *Apidologie* **35**, 133–142.
Schiex, T. and Gaspin, C. (1997). CARTHAGENE: constructing and joining maximum likelihood gene maps. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* **5**, 258–267.
Schneider, S. S. and McNally, L. C. (1993). Spacial foraging patterns and colony energy status in the African honey bee, *Apis mellifera scutellata*. *J. Insect Behav.* **6**, 195–210.
Schulz, D. J., Barron, A. B. and Robinson, G. E. (2002a). A role for octopamine in honey bee division of labor. *Brain Behav. Evolut.* **60**, 350–359.
Schulz, D. J., Pankiw, T., Fondrk, M. K., Robinson, G. E. and Page, R. E. (2004). Comparisons of juvenile hormone hemolymph and octopamine brain titers in honey bees (Hymenoptera: Apidae) selected for high and low pollen hoarding. *Ann. Entomol. Soc. Am.* **97**, 1313–1319.
Schulz, D. J. and Robinson, G. E. (1999). Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. *J. Comp. Physiol. A* **184**, 481–488.
Schulz, D. J., Sullivan, J. P. and Robinson, G. E. (2002b). Juvenile hormone and octopamine in the regulation of division of labor in honey bee colonies. *Horm. Behav.* **42**, 222–231.
Seeley, T. D. (1986). Social foraging by honey bees: how colonies allocate foragers among patches of flowers. *Behav. Ecol. Sociobiol.* **19**, 343–354.
Seeley, T. D. (1995). *The Wisdom of the Hive. The Social Physiology of Honey Bee Colonies*. Cambridge, MA: Harvard University Press.
Seeley, T. D. (1997). Honey bee colonies are group-level adaptive units. *Am. Nat.* **150**, S 22–S 41.
Seeley, T. D., Camazine, S. and Sneyd, J. (1991). Collective decision-making in honey bees: how colonies choose among nectar sources. *Behav. Ecol. Sociobiol.* **28**, 277–290.
Seeley, T.D. and Kolmes, S.A. (1991). Age polyethism for hive duties in honey bees: illusion or reality? *Ethology* **87**, 284–297.
Seeley, T. D., Mikheyev, A. S. and Pagano, G. J. (2000). Dancing bees tune both duration and rate of waggle-run production in relation to nectar source profitability. *J. Comp. Physiol. A* **186**, 813–819.
Sen Sarma, M., Esch, H. and Tautz, J. (2004). A comparison of the dance language in *Apis mellifera carnica* and *Apis florea* reveals striking similarities. *J. Comp. Physiol. A* **190**, 49–53.
Shimizu, I., Kawai, Y., Taniguchi, M. and Aoki, S. (2001). Circadian rhythm and cDNA cloning of the clock gene period in the honeybee *Apis cerana japonica*. *Zool. Sci.* **18**, 779–789.
Smyth, G. K. (2004). Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Gen. Mol. Biol.* **3** Article 3.
Sober, E. and Wilson, D. S. (1998). *Unto Others: The Evolution and Psychology of Unselfish Behavior*. Cambridge, MA: Harvard University Press.
Sokolowski, M. B. (2001). *Drosophila*: genetics meets behaviour. *Nat. Rev. Genet.* **2**, 879–890.
Sokolowski, M. B., Pereira, H. S. and Hughes, K. (1997). Evolution of foraging behavior in *Drosophila* by density-dependent selection. *Proc. Nat. Acad. Sci. USA.* **94**, 7373–7377.

Solignac, M., Vautrin, D., Baudry, E., Mougel, F., Loiseau, A. and Cornuet, J. M. (2004). A microsatellite-based linkage map of the honeybee, *Apis mellifera* L. *Genetics* **167**, 253–262.

Spangler, H. G. (1972). Daily rhythms of individual worker and drone honeybees (*Apis mellifera* L.). *Ann. Entomol. Soc. Am.* **66**, 449–451.

Spangler, H. G. (1973). The role of light in altering the circadian oscillations of the honey bee. *Ann. Entomol. Soc. Am.* **66**, 449–451.

Spivak, M. and Downey, D. L. (1998). Field assays for hygienic behavior in honey bees (Hymenoptera: Apidae). *J. Econ. Entomol.* **91**, 64–70.

Spivak, M. and Gilliam, M. (1998a). Hygienic behaviour of honey bees and its application for control of brood diseases and varroa-Part I. Hygienic behaviour and resistance to American foulbrood. *Bee World* **79**, 124–134.

Spivak, M. and Gilliam, M. (1998b). Hygienic behaviour of honey bees and its application for control of brood diseases and varroa-Part II. Studies on hygienic behaviour since the Rothenbuhler era. *Bee World* **79**, 169–186.

Spivak, M., Masterman, R., Ross, R. and Mesce, K. A. (2003). Hygienic behavior in the honey bee (*Apis mellifera* L.) and the modulatory role of octopamine. *J. Neurobiol.* **55**, 341–345.

Spivak, M. and Reuter, G. S. (1998). Honey bee hygienic behavior. *Am. Bee J.* **138**, 283–286.

Spivak, M. and Reuter, G. S. (2001). Resistance to American foulbrood disease by honey bee colonies (*Apis mellifera*) bred for hygienic behavior. *Apidologie* **32**, 555–565.

Srinivasen, M. V., Zhang, S., Altwein, M. and Tautz, J. (2000). Honeybee navigation: nature and calibration of the ''odometer''. *Science* **287**, 851–853.

Stort, A. C. (1974). Genetic study of the aggressiveness of two subspecies of *Apis mellifera* in Brazil. I. Some tests of aggressiveness. *J. Apic. Res.* **13**, 33–38.

Stort, A. C. and Gonçalves, L. S. (1991). Genetics of defensive behavior II. In: *The ''African'' Honey Bee* (eds Spivak, M. Fletcher, D. and Breed, M.), pp. 329–356. Boulder, CO: Westview Press.

Sullivan, J. P., Fahrbach, S. E., Harrison, J. F., Capaldi, E. A., Fewell, J. H. and Robinson, G. E. (2003). Juvenile hormone and division of labor in honey bee colonies: effects of allatectomy on flight behavior and metabolism. *J. Exp. Biol.* **206**, 2287–2296.

Sullivan, J. P., Jassim, O., Fahrbach, S. E. and Robinson, G. E. (2000). Juvenile hormone paces behavioral development in the adult worker honey bee. *Horm. Behav.* **37**, 1–14.

Take-uchi, M., Kobayashi, Y., Kimura, K. D., Ishihara, T. and Katsura, I. (2005). FLR-4, a novel serine/threonine protein kinase, regulates defecation rhythm in *Caenorhabditis elegans*. *Mol. Biol. Cell.* **16**, 1355–1365.

Tautz, J., Rohrseitz, K. and Sandeman, D. C. (1996). One-strided waggle dance in bees. *Nature* **382**, 32.

Theraulaz, G., Bonabeau, E. and Deneubourg, J.-L. (1998). Response threshold reinforcement and division of labour in insect societies. *Proc. R. Soc. Lond. B* **265**, 327–332.

Thompson, G. J., Kucharski, R., Maleszka, R. and Oldroyd, B. P. (2006). Towards a molecular definition of worker sterility: differential gene expression and reproductive plasticity in honey bees. *Ins. Mol. Biol.* **15**, 637–644.

Toma, D. P., Bloch, G., Moore, D. and Robinson, G. E. (2000). Changes in period mRNA levels in the brain and division of labor in honey bee colonies. *Proc. Nat. Acad. Sci. USA* **97**, 6914–6919.

Trumbo, S. T., Huang, Z. Y. and Robinson, G. E. (1997). Division of labor between undertaker specialists and other middle-aged workers in honey bee colonies. *Behav. Ecol. Sociobiol.* **41**, 151–163.
Van Ooijen, J. W. (2004). *MapQTL 5, Software for the mapping of quantitative trait loci in experimental populations*. Wageningen, Netherlands: Kyazma, B.V..
Vasemagi, A. and Primmer, C. R. (2005). Challenges for identifying functionally important genetic variation: the promise of combining complementary research strategies. *Mol. Ecol.* **14**, 3623–3642.
Vaughan, D. M. and Calderone, N. W. (2002). Assessment of pollen stores by foragers in colonies of the honey bee, *Apis mellifera* L. *Insectes Soc.* **49**, 23–27.
Visscher, P. K. (1989). A quantitative study of worker reproduction in honey bee colonies. *Behav. Ecol. Sociobiol.* **25**, 247–254.
Visscher, P. K. and Seeley, T. D. (1982). Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology* **63**, 1790–1801.
von Frisch, K. (1967). *The Dance Language and Orientation of Bees*. Cambridge, MA: Harvard University Press.
von Frisch, K. and Lindauer, M. (1954). Himmel und Erde in Konkurrenz bei der Orientierung der Bienen. *Naturwissenschaften* **41**, 245–253.
Wahl, O. (1932). Neue Untersuchungen über das Zeitgedåachtnis der Bienen. *Z. Vergl. Physiol.* **16**, 529–589.
Weidenmüller, A. (2004). The control of nest climate in bumblebee (*Bombus terrestris*) colonies: interindividual variability and self reinforcement in fanning response. *Behav. Ecol.* **15**, 120–128.
Whitfield, C. W., Band, M. R., Bonaldo, M. F., Kumar, C. G., Liu, L., Pardinas, J. R., Robertson, H. M., Soares, M. B. and Robinson, G. E. (2002). Annotated expressed sequence tags and cDNA microarrays for studies of brain and behavior in the honey bee. *Genome Res.* **12**, 555–566.
Whitfield, C. W., Cziko, A. M. and Robinson, G. E. (2003). Gene expression profiles in the brain predict behavior in individual honey bees. *Science* **302**, 296–299.
Wilson, E. O. (2000). *Sociobiology: The New Synthesis (25th Anniversary Edition)*. Cambridge, MA: Harvard University Press.
Wilson, E. O. and Hölldobler, B. (2005). Eusociality: Origin and consequences. *Proc. Natl. Acad. Sci. USA* **102**, 13367–13371.
Winston, M. L. (1987). *The Biology of the Honeybee*. Cambridge, MA: Harvard University Press.

## Online links

Honey Bee Genome Project. http://www.hgsc.bcm.tmc.edu/projects/honeybee/
BeeBase. http://www.racerx00.tamu.edu/bee_resources.html
Behaviour and Genetics of Social Insects lab. http://www.bio.usyd.edu.au/Social_InsectsLab/Social_InsectsLab.htm

# Physiological Diversity in Insects: Ecological and Evolutionary Contexts

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## 1 Introduction

Understanding environmental variability and the ways in which organisms respond to such variability over short- and long timescales is of considerable importance to the field of evolutionary physiology, and more

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generally to ecology and to conservation biology. This is as true for insects as it is for other organisms (Prosser, 1986; Spicer and Gaston, 1999; Chown and Nicolson, 2004), and these topics form the substance of this review. After sketching the modern ecological and evolutionary contexts within which evolutionary physiology must now be done, and providing a survey of sources of environmental variability and their effects on insect populations, we move on to explore environmental variation and the various ways in which it may be quantified. Some environmental variables are relatively simple and straightforward, both to measure and to control, whereas others pose substantially greater problems from both perspectives. Even variables that are seemingly easy to measure might act in ways that are difficult to identify (Stenseth and Mysterud, 2005).

Next we briefly revisit definitions of phenotypic plasticity and acclimation. Given their significance it is not surprising that these issues have enjoyed considerable attention over the last decade (e.g. Huey and Berrigan, 1996; Huey *et al*., 1999; Relyea, 2002; Wilson and Franklin, 2002; Piersma and Drent, 2003; West-Eberhard, 2003; DeWitt and Scheiner, 2004; Pigliucci, 2005; Angilletta *et al*., 2006), and in many cases remain the subject of controversy.

Then we examine insect responses to the thermal environment over a variety of spatial and temporal scales, focussing on recent developments in the field. In doing so, we do not suggest that other abiotic or biotic features of the environment (such as water loss, solar radiation, wind, landscape structure, and species interactions) are insignificant. Indeed, the importance of water availability for insect survival and the determination of distribution and abundance patterns has been widely demonstrated (see Hadley, 1994; Tauber *et al*., 1998; Addo-Bediako *et al*., 2001; Hawkins *et al*., 2003; Chown and Nicolson, 2004). Rather, we examine thermal aspects of the environment because they are of considerable significance in determining large- and small-scale patterns of diversity at several scales (Andrewartha and Birch, 1954; Chown and Gaston, 1999; Allen *et al*., 2002; Hawkins *et al*., 2003; Willig *et al*., 2003; Chown *et al*., 2004a; Evans *et al*., 2005).

Finally, we return to the question of what lessons insect evolutionary physiologists might have to offer ecology and conservation biology. In particular, we consider how evolutionary physiology can offer ecologists a set of useful general rules in some cases and can unveil the nature of local contingency in others (see Lawton, 1992, 1999; Chown and Nicolson, 2004; Simberloff, 2004). Although migration ability has a significant influence on the evolution of environmental responses, we do not discuss the costs of flight and the physiology of wing polymorphism and its environmental determinants here (see Zera and Denno, 1997; Shiga *et al*., 2002; Zhao and Zera, 2002, 2004a,b; Cadet *et al*., 2003; Zera and Zhao, 2003 for access to this literature).

## 2 Evolutionary physiology in a changing world

### 2.1 HUMANS AND ECOLOGICAL CHANGE

Humans are altering the modern environment in several ways that affect biodiversity. Most noteworthy among these are habitat destruction and alteration, changes to global, and consequently local climates, pollution (including nutrient enrichment), and the introduction of species to areas from which they were previously absent and in which they subsequently become invasive (Mack *et al*., 2000; Sala *et al*., 2000; Tilman *et al*., 2001; Gaston *et al*., 2003; Palmer *et al*., 2004; Thomas *et al*., 2004; Millenium Ecosystem Assessment, 2005). All of these processes have brought about substantive changes to populations, either by causing local increases or declines in abundance, by promoting changes to life history characteristics so affecting birth and/or death rates, or by affecting rapid local extirpations or introductions. In some cases, these have led to extinction of all populations of some species.

Climate change has resulted in the colonization of higher latitude areas and the establishment of new populations in several northern-hemisphere insect species. This has resulted in substantial range shifts (Parmesan *et al*., 1999), although changes in both range size and position have depended on interactions between the life-history characteristics and habitat requirements of the species concerned, and landscape structure (Hill *et al*., 1999; Thomas *et al*., 2001; Parmesan and Yohe, 2003; Root *et al*., 2003; Simmons and Thomas, 2004; Hill *et al*., 2006). In many cases, climate change effects are negative and have either resulted in or are predicted to give rise to species extinctions (Thomas *et al*., 2004; Pounds *et al*., 2006). Habitat destruction and alteration have likewise substantially affected populations, sometimes changing the entire structure of local assemblages, with subsequent downstream effects on ecosystem functioning (e.g. Steenkamp and Chown, 1996; Cunningham, 2000; Donaldson *et al*., 2002; Rickman and Connor, 2003; Stefanescu *et al*., 2004; Samways *et al*., 2005). The intentional (e.g. for biological control) or accidental introduction of individuals to an area from which they were previously absent has also led to substantial population changes. In the case of the introduced species, new populations are typically established and subsequently increased in abundance (e.g. Dennill *et al*., 1993; Ernsting, 1993; Moller, 1996; McGeoch and Wossler, 2000; Tsutsui *et al*., 2000), while resident, often indigenous, populations are negatively affected (Chown and Smith, 1993; O'Dowd *et al*., 2003; Sanders *et al*., 2003; Holway and Suarez, 2006). The effects of introductions can often be subtle initially, with more pronounced impacts accumulating slowly through time (Chown and Block, 1997; Ernsting *et al*., 1999; Goulson, 2003; Ness, 2004). Nonetheless, in many systems, introductions have resulted in species extinctions (Blackburn *et al*., 2004),

and substantial changes to system functioning (Mooney and Hobbs, 2000; Hansen *et al*., 2002; Goulson, 2003; O'Dowd *et al*., 2003; Blancafort and Gómez, 2005). Finally, the effects of pollution on populations have long been appreciated by freshwater ecologists (see reviews in McGeoch, 1998, in press). However, the sheer pervasiveness and substantial effects of pollution, and especially those of nutrient enrichment, are only now beginning to be appreciated (Millennium Ecosystem Assessment, 2005).

In some instances, the impacts of these processes are likely to be mediated directly by biotic interactions, with only a minimal role played by the abiotic environment. Habitat destruction can lead directly to the loss of populations and species owing to absence of appropriate resources (Brooks *et al*., 1999, 2002; Beier *et al*., 2002; Dunn, 2005), and co-extinctions can exacerbate these impacts (Koh *et al*., 2004). Habitat alteration can cause mesopredator release, thus having knock-on effects on other trophic levels (Crooks and Soulé, 1999), and similar outcomes for particular populations have been documented following invasive species eradication or control efforts (Zavaleta *et al*., 2001). Following climate change or habitat destruction, the incidence of disease can increase, benefiting the disease and, where it is vector-borne, disease vectors, but typically not the host(s) (Patz *et al*., 2000; Harvell *et al*., 2002; Kutz *et al*., 2005; Vittor *et al*., 2006). The opposite situation has also been demonstrated (e.g. Randolph and Rogers, 2000), and is thought to be one of the major ways in which autonomous control of tsetse and trypanosomiasis will be affected (Rogers and Randolph, 2002). Similarly, the effects of invasive alien species on indigenous populations is often direct, either by way of herbivory, predation, or parasitism (Chapuis *et al*., 1994; Mack *et al*., 2000; Blackburn *et al*., 2004), or as a consequence of competition, although the role of invasive species as 'drivers' or 'passengers' in the latter case has yet to be fully resolved (Didham *et al*., 2005).

However, in many situations, impacts on populations of the above mentioned processes have been or will be a direct consequence of changes in the abiotic environment, or have taken place via indirect effects of abiotic factors on other species. This is certainly true of climate change (Bale *et al*., 2002; Walther *et al*., 2002; Root *et al*., 2003). It is well established that the thermal and hygric environments encountered by animals have direct effects on survival, growth, and reproduction (Tauber *et al*., 1998; Denlinger *et al*., 2001; Hochachka and Somero, 2002; Chown and Nicolson, 2004; Kozłowski *et al*., 2004). Nutrient availability, which is being altered by global changes in $CO_2$ and tropospheric ozone levels, also plays a significant role in influencing insect life histories and population dynamics (Slanksy and Rodriguez, 1987; Fagan *et al*., 2002; Woods *et al*., 2003). Likewise, predator–prey and plant–insect interactions can be influenced substantially by the conditions of the abiotic environment (Park, 1962; Chase, 1996; Davis *et al*., 1998; Coviella and Trumble, 1999; Karnosky *et al*., 2003).

Many of the effects of habitat alteration and pollution, and of species introductions are either being realized in similar ways to those described or are substantially influenced by the conditions of the abiotic environment. Habitat destruction has considerable effects on the abiotic environment, which in turn affects population dynamics. Indeed, the coupling between climate and vegetation is well established (Bonan, 2002). For example, in the Atlantic forest region of south-eastern Brazil, a strong positive relationship exists between tree cover and rainfall, indicating that anthropogenic deforestation has resulted in reductions in rainfall (Webb *et al.*, 2006). Small forest patches are likely to suffer further degradation owing to local climate responses to landscape alteration. Large-scale, historical deforestation for agriculture in the United States cooled the climate and led to an increase in the incidence of frost (Bonan, 1999, 2002). These abiotic changes have had large effects on species resident in the landscape.

Perhap, one of the most striking examples of the effects of land use change on insect mortality, via changes in abiotic conditions, is the case of Monarch butterflies overwintering in oyamel fir forests in Mexico. The adult butterflies are susceptible to freezing (freezing and dying at c. $-8.7\,^{\circ}C$), especially by inoculation if they become wet (freezing at c. $-3.7$–$-4.5\,^{\circ}C$) (Alonso-Mejía *et al.*, 1992; Larsen and Lee, 1994). Forest cover not only forms an umbrella offering protection from direct rainfall, but it also prevents windblown spray from reaching the butterflies (Anderson and Brower, 1996). Clustering by butterflies in the forest promotes retention of high lipid reserves (Alonso-Mejía *et al.*, 1997), and a well-developed understory enables adults that have been knocked from clusters to regain height, so avoiding dew and benefiting from the aggregations (Alonso-Mejía *et al.*, 1992, 1997). Forest thinning and understory removal, as a consequence of human activities, therefore poses substantial threats to these butterflies by increasing overwintering mortality. Global climate change forecasts suggest that cool-weather precipitation is likely to increase in the overwintering sites, thus bringing additional risk, especially if forest cover is thinned. These changes will render many present sites wholly unsuitable within 50 years (Oberhauser and Peterson, 2003). Other studies have demonstrated effects of microclimate changes on insect assemblages (e.g. Perfecto and Vandermeer, 1996).

The likelihood of establishment and subsequent spread of a species alien to a given area is, at least to some extent, a function of the interaction between individuals of that species and the abiotic environment. It is widely appreciated that a match in climate between native and introduced ranges is a reasonable, though not the only or a foolproof (see Samways *et al.*, 1999), predictor of success of an alien species in its introduced range, whether the species is an unintentional introduction, or a biological control agent (e.g. Dennill and Gordon, 1990; Duncan *et al.*, 2003; Robertson *et al.*, 2004). Similarly, both productive and ambient energy are strong correlates of broad-scale variation in alien species richness (Chown *et al.*, 2005; Richardson *et al.*, 2005).

These examples clearly illustrate that comprehension of human impacts on modern diversity requires an understanding of the effects of the abiotic environment on individuals and populations (of different species), and the ways in which individuals and populations respond to the environment and its spatial and temporal variation. Such knowledge is also necessary for predicting what interventions might be required given a future of ongoing change (Hannah *et al.*, 2002; Walther *et al.*, 2002; Williams *et al.*, 2005; Xenopoulos *et al.*, 2005). While several bioclimatic modelling approaches (see Pearson and Dawson, 2003; Huntley *et al.*, 2004; Segurado and Araújo, 2004) are available that provide a first, and much-needed, estimate of likely species abundances and occurrences (Rogers and Randolph, 1991; Jeffree and Jeffree, 1996; Robinson *et al.*, 1997a,b; Randolph and Rogers, 2000; Rogers, 2000; Erasmus *et al.*, 2002; Pearson and Dawson, 2003; Tatem *et al.*, 2003; Huntley *et al.*, 2004; Thomas *et al.*, 2004), they are based almost solely on climatic correlates of abundance and distribution, and have, in consequence, been criticized (e.g. Davis *et al.*, 1998; Samways *et al.*, 1999). From a physiological perspective, concerns have come from three principal perspectives. Spatial variation in population responses to the environment is often not considered (Davis and Shaw, 2001); the rapid alterations to phenotypes that might take place via phenotypic plasticity in the form of developmental plasticity, acclimation, and hardening are typically ignored (Helmuth *et al.*, 2005); and the likely outcome of covariation among abiotic variables, and their interaction with other components of the environment, such as risk of predation and intensity of competition, are often not adequately assessed (Rogers and Randolph, 2000; Angilletta *et al.*, 2006). Spatial and temporal variability in phenotypes might substantially alter predicted responses to change (Stillman, 2003), especially if this variability varies among traits (Chown, 2001; Hoffmann *et al.*, 2003a). Consequently, it has been proposed that physiological investigations and biophysical modelling should be used in concert with large-scale bioclimatic investigations of species responses to understand what the future might hold for various taxa in a climate of change (Helmuth *et al.*, 2005). Thus, it is clear that evolutionary physiologists face substantial challenges, not only in deepening understanding of how organisms respond to their changing environments, but also in addressing the demands being made of them by ecologists and conservation biologists concerned about the appropriate actions to take in the face of rapid, global environmental change (Angilletta *et al.*, 2006; Wikelski and Cooke, 2006).

## 2.2 VARIABILITY AND CHANGE IN POPULATIONS

Physiological responses to changes in the environment take place over a range of time scales, from rapid, phenotypic adjustments to longer-term, evolutionary changes that might also alter the phenotypic response to the

environment (Hochachka and Somero, 2002; West-Eberhard, 2003; Chown and Nicolson, 2004). The likelihood that one or more of these responses will be realized depends on the nature of the environment in which the population finds itself, and the extent to which the population is connected to others by dispersal, whether or not this dispersal takes place in a metapopulation landscape.

Physiologists have long appreciated that environmental conditions and their variability have an influence on phenotypic plasticity (see Section 4). It is widely thought that acclimatization is more likely in species from temperate than those from less variable tropical and polar environments (Spicer and Gaston, 1999; Ghalambor *et al.*, 2006), and less likely in stenothermal (narrow temperature tolerance) species (Somero *et al.*, 1996; Pörtner *et al.*, 2000), although tropical species might be more eurythermal (wide temperature tolerance) than their polar counterparts (Somero, 2005). More generally, the environmental circumstances under which adaptive population differentiation, phenotypic plasticity, or some combination thereof arise form the subject of a large and growing theoretical field (e.g. West-Eberhard, 2003; Berrigan and Scheiner, 2004; Pigliucci, 2005). Somewhat surprisingly, this field and work examining the evolution of thermal physiology remain reasonably distinct (though see Lynch and Gabriel, 1987; Gilchrist, 1995), even though the physiological models often struggle to explain the high frequency of eurythermic strategies (see reviews in Angilletta *et al.*, 2002, 2003, 2006). Hence, we focus on the former plasticity models, noting parallels with the thermal physiology models where appropriate.

Many investigations have shown that greater environmental variability tends to favour phenotypic plasticity within populations, as long as cue reliability and accuracy of the response (which is a function of environmental lability and unpredictability, and of the extent to which the response lags behind the environmental change) is high, and the cost of plasticity is low (Lively, 1986; Moran, 1992; Scheiner, 1993; Tufto, 2000). This conclusion holds for both optimality and quantitative genetic (environmental threshold) models (Hazel *et al.*, 2004). Recent modelling work has also shown that the likelihood of this outcome is affected strongly by migration between different populations (Tufto, 2000; Sultan and Spencer, 2002). With little or no migration, and different environments, adaptive differentiation between populations in each of these environments readily evolves. Increases in migration rate, by contrast, lead to fixation of the plastic phenotype even though it might not be the best type anywhere (i.e. relative to adaptively differentiated habitat specialists) (Tufto, 2000; Sultan and Spencer, 2002). Nonetheless, if response accuracy is low (i.e. no better than random for at least one environmental state), the specialist phenotype is favoured, and the same is likely to be true if the global cost of plasticity is high (though evidence for the latter is scarce) (Van Tienderen 1991, 1997;

Moran 1992; Sultan and Spencer, 2002, but see also Relyea 2002; van Kleunen and Fischer, 2005). In addition, environmental-threshold models show that with low cue reliability and low frequency of benign patches, a reversed (counter–intuitive) conditional, but unstable, strategy is favoured (Hazel *et al*. 2004).

In the context of insect physiological responses, four outcomes of these models are most notable. First, plastic responses are likely to be common across a broad range of conditions in the presence of even relatively low levels of migration. Second, plastic phenotypes might be favoured globally even when in any given environment they have a lower fitness than a habitat specialist (Sultan and Spencer, 2002). The plastic responses might also be the reverse of what is expected under a given set of circumstances (Hazel *et al*., 2004). Third, variation in trait response lag times, such as between developmental change and acclimation (see Section 4), might account for differences in plasticity among traits. Finally, these outcomes will be affected by the level of within-site homogeneity, the number of sites in any given broader environment, and the frequency of different kinds of patches. In insects, populations connected by migration (whether or not a true metapopulation system is demonstrated – e.g. Harding and McNamara, 2002) are relatively common (see Schneider, 2003; Roslin and Kotze, 2005 for discussion), suggesting that plasticity will be regularly found in many traits and might account for a substantial proportion of the 'population differentiation' found between them. Evidence is accumulating that this is indeed the case, as reflected in recent assessments of the contribution of plasticity to population variation in thermal tolerance traits of several taxa, including *Drosophila* (Ayrinhac *et al*., 2004; Hoffmann *et al*., 2005a), weevils (Klok and Chown, 2003), and tsetse (Terblanche *et al*., 2006). Significantly, in the case of the weevils, the single widespread species investigated, which is present on two islands separated by thousands of kilometres, thus precluding dispersal, showed substantial inter-island population differentiation in lower thermal limits that could not be accounted for by phenotypic plasticity, in keeping with theoretical predictions. These findings also suggest that genetic accommodation (also more narrowly thought of as epigenetic assimilation) has been significant in the evolution of thermal tolerances in insects (see Pigliucci and Murren, 2003; West-Eberhard, 2003).

If plastic phenotypes are favoured globally, even if their fitness is not highest at any particular site, then negative tests of the beneficial acclimation hypothesis under a particular set of conditions might not be unexpected. Together with the tendency for many tests of beneficial acclimation to focus on developmental changes (which might be less likely to be demonstrably beneficial because of response lag times) (Wilson and Franklin, 2002), this might account for recent conclusions that acclimation (a form of plasticity) is typically not beneficial (Huey *et al*., 1999; Wilson

and Franklin, 2002), despite the fact that theoretical models demonstrate a wide range of scenarios under which adaptive phenotypic plasticity might evolve.

### 2.3 DISPERSAL, PLASTICITY, AND RANGE EDGES

That population connectivity has a strong influence on the likelihood of local adaptation has also been recognized in the context of the mechanisms determining species range margins (Hoffmann and Blows, 1994; Lenormand, 2002). While the proximate determinants of range margins might appear to be the inability of a population to cope with a given set of circumstances that lie just beyond its range, the ultimate determinants of range margins have to do with the inability of a population to respond to these circumstances, which it must do to achieve either colonization, population growth, or stasis (Carter and Prince, 1981; Gaston, 2003). Populations might be unable to persist in a given area as a consequence of an absence of suitable habitat patches, an increase in extinction rate such that population persistence is impossible (i.e. reflecting lack of adaptation in the broadest sense), or a decline in dispersal or colonization rate (Holt and Keitt, 2000). In other words, gradients in any of these factors might result in range margins. Why a population should be unable to adapt to local circumstances beyond its range, thus reducing extinction probability, increasing colonization success, or enabling a change in habitat use, is thought to be a result of low genetic variation, low heritability, genetic trade-offs, mutation accumulation, and the need for changes in several components of the phenotype simultaneously (Hoffmann and Blows, 1994; Gaston, 2003; Blows and Hoffmann, 2005). It is also thought to be a result of swamping of genotypes in marginal populations by those from central populations via immigration (Case and Taper, 2000; Gaston, 2003; Alleaume-Benharira *et al*., 2006). In other words, gene flow inhibits local adaptation. Kirkpatrick and Barton (1997) showed that random dispersal results in a flow of genes to the periphery of the species' range so turning peripheral populations into 'sinks' where death rates are higher than birth rates. Paradoxically, while gene flow maintains the number of individuals, it also has the effect of ensuring that the peripheral population remains a sink. Nonetheless, in several situations a balance is struck between local adaptation and gene flow, which then sets a species' range limits. This balance can be altered by relatively small changes in the parameters in Kirkpatrick and Barton's (1997) model, thus explaining the rapid expansions of populations that are sometimes seen. Subsequent modelling work has shown that if sink populations are variable and this variation is temporally autocorrelated (as is the case of almost all abiotic variation), then adaptation in peripheral populations can take place even in the face of gene flow (Holt *et al*., 2004a). In essence, a favourable period may lower the

extent of maladaptation in sink environments for long enough to allow population growth, which in turn would reduce the effects of gene flow from immigrants. Low levels of migration also mitigate the negative effects of genetic drift and may reduce stochastic variation around the mean phenotype that is the consequence of drift (Alleaume-Benharira *et al.*, 2006). In consequence, depending on population size and the strength of the environmental gradient, the optimal migration rate (see Alleaume-Benharira *et al.*, 2006) is an intermediate one (see also Forde *et al.*, 2004; Holt *et al.*, 2004b).

Typically, models of the influence of gene flow on range limits have not considered the simultaneous influence of migration on the evolution of phenotypic plasticity. Kirkpatrick and Barton (1997) acknowledged that the tendency for gene flow to swamp local adaptation might be ameliorated by phenotypic plasticity, but did not take the matter further. Likewise, Holt *et al.* (2004a) gave no attention to the likelihood that instead of promoting local adaptation, autocorrelated environmental variability (which would improve response accuracy) is likely to promote the evolution of phenotypic plasticity. In consequence, it is difficult to ascertain what the influence of phenotypic plasticity on the evolution of range edges might be (see also Sultan, 2004). On the one hand, it might promote local adaptation of a kind by allowing populations to persist (Kirkpatrick and Barton, 1997), and perhaps to grow out of the substantial effects of gene flow on local adaptation (Holt *et al.*, 2004a,b; see also West-Eberhard, 2005). On the other hand, it seems equally likely that phenotypic plasticity might prevent local adaptation because it is the favoured strategy everywhere, despite lower fitness in some locations (Sultan and Spencer, 2002). This would frustrate local adaptation and prevent range expansion. Clearly, there is a need to link models investigating the effects of migration on local adaptation (Kirkpatrick and Barton, 1997; Holt *et al.*, 2004a), and those investigating the conditions that promote phenotypic plasticity (Tufto, 2000; Sultan and Spencer, 2002). This amounts to an understanding of the role of genetic accommodation in setting and/or altering range limits (see Pigliucci and Murren, 2003; West-Eberhard, 2003; Pigliucci *et al.*, 2006).

### 2.4 IMPLICATIONS FOR INSECT PHYSIOLOGY

Models of the kinds described provide considerable insight into the significance of phenotypic plasticity for mediating species responses to environmental change. Thus, not only is it important to understand the extent to which various traits show phenotypic plasticity, but it is also important to comprehend the conditions that promote such variability relative to changes in basal responses. It is not just understanding of the ways in which populations might avoid extinction that can be informed by such investigations. Recently, Wiens (2004) has argued that comprehension

of the reasons why populations are unable to expand their ranges is likely to provide considerable insight into what causes new lineages to arise – i.e. what is the cause of speciation in allopatry. Understanding what traits determine the inability of species to occupy certain habitats (and these are often likely to be physiological (Gaston, 2003; Wiens, 2004)), and why these show little capacity for change in some instances and considerable capacity for change in others (West-Eberhard, 2003) is therefore significant in the context of both extinction and speciation, the ultimate determinants of species richness variation on the planet (West-Eberhard, 1989; Gaston and Chown, 1999; Chown and Gaston, 2000).

## 3 Abiotic environmental variation and its measurement

That weather and climate have significant effects on insect populations has long been appreciated by ecologists (Shelford, 1911; Andrewartha and Birch, 1954; Messenger, 1959; Kingsolver, 1989; Roy *et al*., 2001). The coincidence of species range edges with particular climatic features (Chown and Gaston, 1999), robust relationships between climatic variables and insect abundances and distributions (Jeffree and Jeffree, 1996; Robinson *et al*. 1997a,b), and the recent response of species range edges to global climate change (Parmesan *et al*., 1999), all serve to emphasize that climate exerts a significant effect on insect populations. Fluctuations in abundance through time, as a consequence of changes in birth rates, death rates or both, in synchrony with changes in weather, similarly highlight the significance of weather for the population dynamics of many insect species (Andrewartha and Birch, 1954; Kingsolver, 1989; Roy *et al*., 2001; Hargrove, 2004). Appreciation for the fact that microclimatic measurements are of considerable importance for understanding insect responses to the environment is also well developed (Willmer, 1982; Leather *et al*., 1993; Danks, 1996, 1999; Hodkinson, 2003). More recently, the emphasis of investigations has shifted to variability and unpredictability (Kingsolver and Huey, 1998; Angilletta *et al*., 2006; but see also Levins, 1968), the intensity of extreme conditions (e.g. Gaines and Denny, 1993; Parmesan *et al*., 2000), and the frequency, rate of approach to, and duration of particular conditions (Sømme, 1996; Kelty and Lee, 1999; Sinclair, 2001a; Robertson, 2004a; Rako and Hoffmann, 2006).

### 3.1 MEANS AND EXTREMES

Owing to their availability, even before the advent of widely available remotely sensed information and geographic information systems, data on the annual means (e.g. of temperature) or totals (e.g. precipitation) of Stevenson Screen data across broad geographic scales were regularly

used as independent variables for examination of large-scale variation in physiological traits. Recent studies have adopted similar approaches, documenting significant and sometimes strong relationships between the variables of interest and the climatic parameter used (see e.g. Addo-Bediako *et al.*, 2001, 2002; Hoffmann *et al.*, 2003b; Parkash *et al.*, 2005).

The use of mean annual climatic data has proven controversial, however. It has been argued that insects are unlikely to experience these mean temperatures because of microhabitat selection and inactivity of certain stages at particular times of the year (see e.g. Hodkinson, 2003). Undoubtedly this is true, as many studies have demonstrated (see Kevan, 1975; Bale, 1987; Leather *et al.*, 1993; Sinclair and Chown, 2005a, for examples). However, the crux of the matter lies in the question being posed and the scale at which it is investigated. For large-scale, comparative studies it is unlikely that microclimate data will be available for every site from which the study organisms have been collected. Many individual studies simply provide a locality name and a broad description of prevailing local climates, and if climatic data are provided they are often supplied from the nearest meteorological station (i.e. Stevenson Screen values). Anyone interested in large-scale patterns in variation must then come to a decision about what parameters to use, and 'macroclimatological' variables are certainly more informative than none at all (see Chown *et al.*, 2003). In addition, these can be useful in revealing the likely cause of variation in a given biological variable. For example, along the east coast of Australia, highest daily maximum temperature in the hottest month does not vary with latitude, but mean daily maximum temperature declines with latitude. Thus, the number of warm days declines as latitude increases and this variation is probably the cause of clinal variation of the 56H8 heat-shock protein (hsp70) allele in *Drosophila melanogaster* (Bettencourt *et al.*, 2002).

It has been argued that large-scale studies are perhaps of little value because of uncertainties associated both with the microhabitats occupied by the species (or populations) and the biology of the species concerned (Hodkinson, 2003). Such tension between broader-scale and finer-scale approaches is not new (e.g. Feder, 1987), and has been discussed in detail recently in the context of population dynamics and community ecology (Lawton, 1992, 1999; Simberloff, 2004). In our view, both approaches have their merits and drawbacks, and each reveals patterns and mechanisms that would simply have remained undetected had the approach not been followed (see also Chown *et al.*, 2004b). For example, it is only through local-scale work, with fine temporal resolution, that the responses of the goldenrod gall fly, *Eurosta solidaginis*, to its thermal and hygric environments have come to be comprehended (Storey *et al.*, 1981; Storey and Storey, 1986; Storey, 1990; Joanisse and Storey, 1994a; Lee *et al.*, 1995; Irwin and Lee, 2000; Williams *et al.*, 2004). And it is this work that is enabling novel insights to be gained into the role of hypoxia-inducing-factor-1$\alpha$

in mediating resistance to cold, freezing, and anoxia (Morin *et al.*, 2005). By contrast, in the absence of large-scale work, it would not have become clear that over broad spatial scales, upper lethal limits are much less variable than lower lethal limits (Addo-Bediako *et al.*, 2000; Gibert and Huey, 2001; Kimura, 2004). Nor would it be apparent that substantial differences exist between the high latitude northern and southern hemispheres in the cold hardiness strategies adopted by insects, and that these differences may be driven in part by differences in unpredictability of freezing events owing to the 'mean' climates of the two hemispheres (Sinclair *et al.*, 2003a; Sinclair and Chown, 2005a).

Several recent studies, especially of mammalian and avian population dynamics and life histories, have shown why measured variables such as temperature and precipitation, might be much less adequate at explaining population responses than broader climate indices such as the North Atlantic Oscillation (NAO) or El Niño Southern Oscillation (ENSO) (Hallett *et al.*, 2004; Stenseth and Mysterud, 2005). In essence, by integrating a variety of weather variables across spatial and temporal scales that are of significance for the animals, these indices often provide a much better estimate of the overall quality of a season than do short-term measurements such as temperature or snowfall at a given site for specific months (Fig. 1). In consequence, where specific climatic variables differ from year to year in their relationship with aspects of population dynamics, and fail to capture the complexities of environmental effects on animals (such as

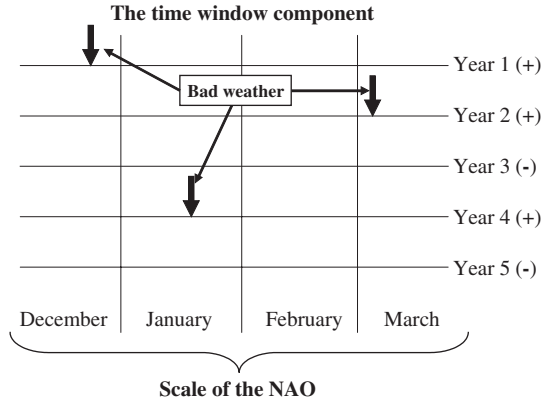



FIG. 1 Climate indices such as the North Atlantic Oscillation (NAO) integrate a variety of weather variables across spatial and temporal scales. Here, poor weather in years two, three, and four takes place in different months. However, the sign of the climate index (in this case NAO) indicates that these years have been poor irrespective of when the worst conditions have been experienced. Redrawn from Stenseth and Mysterud (2005, p. 1196) with permission from the British Ecological Society.

the importance of combinations of variables such as low temperature and high precipitation – see the discussion of monarch butterfly mortality in Alonso-Mejía *et al*., 1992), the climate indices are effective in representing the overall quality of a season and so can explain much of the variation in population responses. Therefore, these indices can also provide substantial predictive capability and an indication of longer-term change associated with changing broad-scale climatic patterns (Stenseth and Mysterud, 2005). While the relationships between climatic indices (sometimes called teleconnection patterns) and insect responses have not been fully explored, a growing number of studies indicate that these relationships bear closer scrutiny (e.g. Holmgren *et al*., 2001; Ottersen *et al*., 2001; Sinclair, 2001a; Gagnon *et al*., 2002; Conrad *et al*., 2003; Briers *et al*., 2004). 'Biologically relevant' guides to these indices and discussion of their relationships with local weather variables are becoming more common, making their use accessible to a wide range of disciplines (Stenseth *et al*., 2003).

To understand the effects of extreme weather on insects and their likely physiological responses, often in anticipation of these extremes, local scale, temporally explicit studies are nonetheless necessary. That extreme abiotic conditions have significant effects on population dynamics, and even population persistence has been demonstrated on several occasions (Leather *et al*., 1993; Roy *et al*., 2001). For example, populations of *Euphydryas editha* (Lepidoptera, Nymphalidae) were driven to extinction as a consequence of three extreme weather events, and human landscape alteration. In one year, minimal snow led to early April (rather than June) emergence of adults and their subsequent starvation owing to an absence of nectar. A year later emergence was once again early for the same reason, and a ''normal'' snowstorm in May resulted in high mortalities. In 1992, unusually low temperatures killed most of the host plants, leaving caterpillars with no source of food (Thomas *et al*., 1996; Parmesan *et al*., 2000). Winter mortality has also been shown to be the source of population (larval) mortality in the butterfly *Atalopedes campestris*, and warming climates have meant enhanced survival of this species and population persistence in some previously uninhabitable areas (Crozier, 2003, 2004).

It is not only the intensity and occurrence of extreme events that are important, but also the duration of the events, the rates at which they are approached, and the likelihood of their occurrence within a given time frame (i.e. their frequency or return time) (Gaines and Denny, 1993; Gutschick and BassiriRad, 2003). The significance of the intensity and duration of stressful conditions, and their interactions, has long been appreciated by physiologists (for discussion see Cossins and Bowler, 1987; Hochachka and Somero, 2002), and continues to attract the attention of insect physiologists (e.g. Sømme, 1996; Nedved, 1998; Shintani and Ishikawa, 1999; Irwin and Lee 2000, 2002; Jing and Kang, 2003; Neargarder *et al*., 2003; Renault *et al*., 2004; Rako and Hoffmann, 2006). Recent work

has shown that sublethal exposures may also have substantial impacts. For example, repeated sublethal exposures to high temperature induce substantial mortality in the flesh fly, *Sarcophaga crassipalpis*, although this thermosensitivity can be overcome by a hardening treatment (Denlinger and Yocum, 1998). In the caterpillars of the tineid moth, *Pringleophaga marioni*, repeated sublethal low-temperature exposures affect gut functioning, thus depressing growth rates relative to control larvae, and in consequence sublethal events have a negative effect on fitness (Sinclair and Chown, 2005b). In the fly, *Syrphus ribesii*, repeated stressful exposures result in substantial mortality and an altered cold hardiness strategy (Brown *et al.*, 2004). Such a change in strategy and effect of repeated stressful, typically sublethal mortality events has also been documented in the beetle, *Hydromedion sparsutum* (Bale *et al.*, 2001).

The rate at which a particular stressful event is approached is important. While early work demonstrated that some variables, such as crystallization temperature (or supercooling point, SCP) are little influenced by changes in rate (Salt, 1966, see also Sinclair *et al.*, 2006), other variables can be profoundly affected. For example, cooling rate may be significant in determining survival of freezing-tolerant insects (Miller, 1978; Shimada and Riihimaa, 1990). In other freezing-tolerant insects, the rate of freezing is important because it affects the likelihood of intra-cellular ice formation (Worland *et al.*, 2004). Cooling rate has a substantial effect on mortality caused by low temperatures and on critical thermal minima because low rates of cooling can provide opportunities for a rapid cold hardening response (Kelty and Lee, 1999, 2001). Likewise, the rate at which conditions return to more benign values is significant, especially following exposure to cold and desiccation, because a return to more normal conditions has profound physiological effects, and might cause stress responses (e.g. Yocum *et al.*, 1991; Joanisse and Storey, 1998; Hayward *et al.*, 2004a; Nielsen *et al.*, 2005).

Recognition of the fact that intensity, duration, and frequency of, rate of approach to, and rate of departure from extreme events all have significant effects on physiological responses and the fitness of insect populations has re-invigorated interest in documenting climate variability in the field. The wide availability of appropriate sensors and datalogging equipment has made such documentation more tractable. Fortunately, a variety of techniques is available for analyzing both more conventional microclimatic data and those relevant for the assessment of extreme values (e.g. Gaines and Denny, 1993; Ferguson and Messier, 1996; Sinclair, 2001b; Vasseur and Yodzis, 2004).

### 3.2 VARIABILITY AND UNPREDICTABILITY

Insects show a variety of behavioural responses to small-scale temporal and spatial variation in abiotic conditions. For example, short-term selection of

sunlit patches is one of the most common mechanisms for regulating body temperature (May, 1979; Dreisig, 1980), which at least in some cases results in a close match between preferred body temperature and body temperatures realized in the field (Ward and Seely, 1996). These responses depend on the mechanisms of physiological regulation open to the species (see also Angilletta *et al.*, 2006 for a vertebrate perspective). In the case of thermoregulation, they differ substantially between species of different size, those capable of basking and those able to generate endogenous heat, and between these species and those that employ neither mechanism (see e.g. Herrera, 1997; Sformo and Doak, 2006; and discussion in Chown and Nicolson, 2004). Evolution of tolerance of extreme conditions may also enable species to make use of resources that are typically unavailable under more benign conditions owing to inter-specific interactions. Such a daily partitioning of the thermal environment is thought to represent one of the mechanisms enabling coexistence of competing ant species (Cerdá *et al.*, 1998; Bestelmeyer, 2000; Parr *et al.*, 2005). The varying regulatory abilities (physiological and/or behavioural) of species also contribute to their apparent daily and seasonal abundances, or variation in activity and phenology (Hodkinson *et al.*, 1996; Danks, 1999; Gordon *et al.*, 2001). For example, during their summer-activity peak, adults of *Bothrometopus brevis* on Heard Island are most active during comparatively warm, north-wind and light-rain conditions (Fig. 2). During heavy rain, activity is low, and it is negligible when low temperatures, associated with south winds, prevail, especially if these are accompanied by snow and sleet (Chown *et al.*, 2004c).

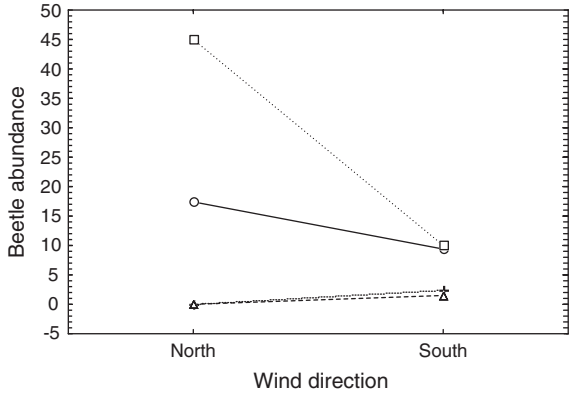



FIG. 2 Interaction plot of mean numbers of adult *Bothrometopus brevis* weevils active at a site on sub-Antarctic Heard Island for each combination of weather conditions prevailing at the site over the course of a summer, including the two major wind directions (north or south) and either no precipitation (○), light rain (□), snow (+), or heavy precipitation of either kind (Δ). Redrawn from Chown *et al.* (2004c).

Relatively poor resistance to desiccation in the group of weevils to which the species belongs is likely responsible for this behaviour and for their tendency to shelter in rock crevices during dry conditions (Chown, 1993; Chown and Klok, 2003).

Perhaps, the most well-investigated responses in insects to varying conditions are those associated with seasonal changes in the environment. Dormancy, and especially the endogenous, centrally mediated change that controls diapause has received the most attention. It is the subject of a large literature that has recently been reviewed from several perspectives. Notable amongst these is the clarification of the ecophysiological phases of insect diapause, and their associated terminology (Koštál, 2006), and an overview of the molecular regulation of diapause (Denlinger, 2002). These reviews also refer to further syntheses of work describing the hormonal and physiological changes that are associated with the major phases of diapause: induction of, preparation for, and the initiation, maintenance, and termination of diapause (Fig. 3). One of the most thoroughly explored of these changes is the development of cold hardiness, or a programmed response to cold (Chown and Nicolson, 2004). Although the development of cold hardiness is not always associated with diapause, the two 'programmes' are often intimately related (Denlinger, 1991, 2002). Insect responses to changing seasonal conditions have been reviewed from a wide variety of perspectives with much emphasis being placed on the seasonal progression of physiological states and their underlying molecular mechanisms (e.g. Leather *et al.*, 1993; Sømme, 1995; Hallman and Denlinger, 1998; Storey, 2002; Storey and Storey, 2004).

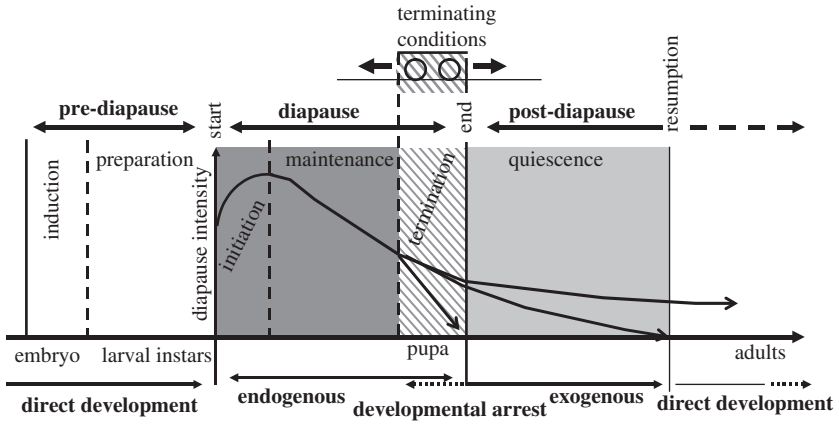



FIG. 3 Schematic depiction of the major phases of diapause, *viz*. pre-diapause, diapause, and post-diapause, as defined by Koštál (2006). Further division into subphases, *viz*. induction, preparation, initiation, maintenance, termination, and quiescence is indicated by vertical lines. Redrawn with permission from Elsevier.

Several recent studies have also indicated that 'unpredictable' or unusual events, associated with inter-annual climatic variability, can have a substantial effect on survival of overwintering insects. For example, unseasonably warm Arctic weather can lead to surface ice formation (following rain rather than the usual snow), resulting in substantial mortality of soil-dwelling species (Coulson *et al.*, 2000). Similarly, in *Eurosta solidaginis*, overwintering at mild temperatures results in a substantial decline in survival as well as in mass of the larvae at the end of the overwintering period (Irwin and Lee, 2000). Mass loss is significant because it translates to a decline in fitness owing to lower fecundity in lighter adult females. The effect of milder temperatures is also reflected in a latitudinal cline in overwinter mass loss, from c. 7.5% in the colder northern parts of the species' range to c. 20% in milder southern areas. At finer spatial scales, galls that remain below the snow experience milder conditions, as a consequence of insulation by the snow and greater warming during spring days, than exposed galls. Larvae in the former consume much of their stored energy, resulting in lower fecundity, and it seems likely that this has resulted in strong selection for overwintering above the snow and substantial freeze tolerance (Irwin and Lee, 2003).

Inter-annual variability in precipitation can have substantial effects on insect-development rates, leading to considerable population variance. In herbivorous caterpillars, this unpredictable variation in growth rates, coupled with the unpredictable variation in climate has substantial effects on parasitoid dynamics. In areas with a high-precipitation coefficient of variation (c.v.), parasitism frequency is low, whereas it is much higher in areas of low precipitation c.v. (Stireman *et al.*, 2005). These effects are much more pronounced in host-specific parasitoids, which have little opportunity for exploiting alternative resources. If this spatial relationship applies through time, then increases in the variability of the abiotic environment, as are predicted to occur in many areas as a component of global environmental change (Watson, 2002), will mean increased caterpillar outbreaks. Such outbreaks have substantial ecosystem effects, cascading through multiple trophic levels, with both economic and conservation implications (e.g. Myers, 1988; Hódar *et al.*, 2003).

Given that unseasonable weather events can have substantial influences on survival, that climatic variation can influence host–parasitoid interactions through differential responses of these groups to abiotic factors, and that both variability and unpredictability are key factors influencing the likely evolution of phenotypic plasticity, documenting variability and unpredictability is of considerable importance. Such documentation must recognize that both of these environmental characteristics vary at a variety of scales (Kingsolver and Huey, 1998), and that the significance of the scale of variation will depend on both the size and longevity of the insect stage that is being investigated. For example, investigation of inter-annual variation and predictability of winter minima is unlikely to be directly

significant for the short-lived adults of an insect species such as a goldenrod gall fly, but might be of considerable indirect significance because such temperatures will determine adult fecundity via energetic effects on the larval stage (see also Angilletta *et al.*, 2006).

Although standard measures of variation, such as differences in seasonal means, minima, and temperature ranges, and coefficients of variation, provide considerable insight into variability in conditions, they are less appropriate for understanding predictability of these conditions. Several approaches allow the latter to be done. One of the most straightforward approaches is to examine correlations of conditions over a variety of temporal scales, which can reveal differences in predictability at the scale of the individual's lifetime, between seasons, and between years (Kingsolver and Huey, 1998). In addition, examination of the autocorrelation plots of the time series in question provide a rapid way of assessing predictability of a particular environmental variable. For example, hourly soil temperatures over a weeklong period at a site on the west coast of South Africa (Lambert's Bay) are perfectly predictable from day to day (Fig. 4a), and this is reflected in significantly positive autocorrelations at lags of 24 h and multiples thereof (Fig. 5a). Although conditions at higher altitudes are a little less predictable (Fig. 4b), a similar autocorrelation pattern can be seen (Fig. 5b). By contrast, sea level and high altitude soil temperatures at sub-Antarctic Marion Island are far less predictable (Fig. 4a, b), as is immediately obvious from the autocorrelation plots. In the sea-level example (Fig. 5c), temperatures are significantly dissimilar to those experienced 24 h previously than would be expected by chance, and at the higher elevation the signal rapidly becomes indistinguishable from white noise (Fig. 5d). Fourier analyses provide similar conclusions, with the South-African site data showing greatest spectral density at 24 h, and the Marion Island site data showing rather weaker signals at 55 h at sea level, and no significant signal for the higher elevation site.

The calculation of spectral densities is being widely applied in ecology as a means of investigating the form and significance of environmental noise for populations and other levels in the ecological hierarchy. The importance of the colour of noise was first raised in an ecological context by Steele (1985) and has since been the subject of much attention (e.g. Lawton, 1988; Halley, 1996; Cohen *et al.*, 1998; Storch *et al.*, 2002). White noise contains an equal mix of all frequencies, with a flat spectral density. It is a special case of a family of noise forms in which variance scales with frequency according to an inverse power law, $1/f^{\beta}$ (Halley, 1996; Vasseur and Yodzis, 2004). In the case of white noise, $\beta = 0$. If the spectral density is greater at low than at high frequencies then the spectrum is said to be reddened: low frequency cycles dominate. Brown noise refers to a signal generated by Brownian process or a random walk. By contrast, pink noise lies midway between brown and white noise. In a comprehensive

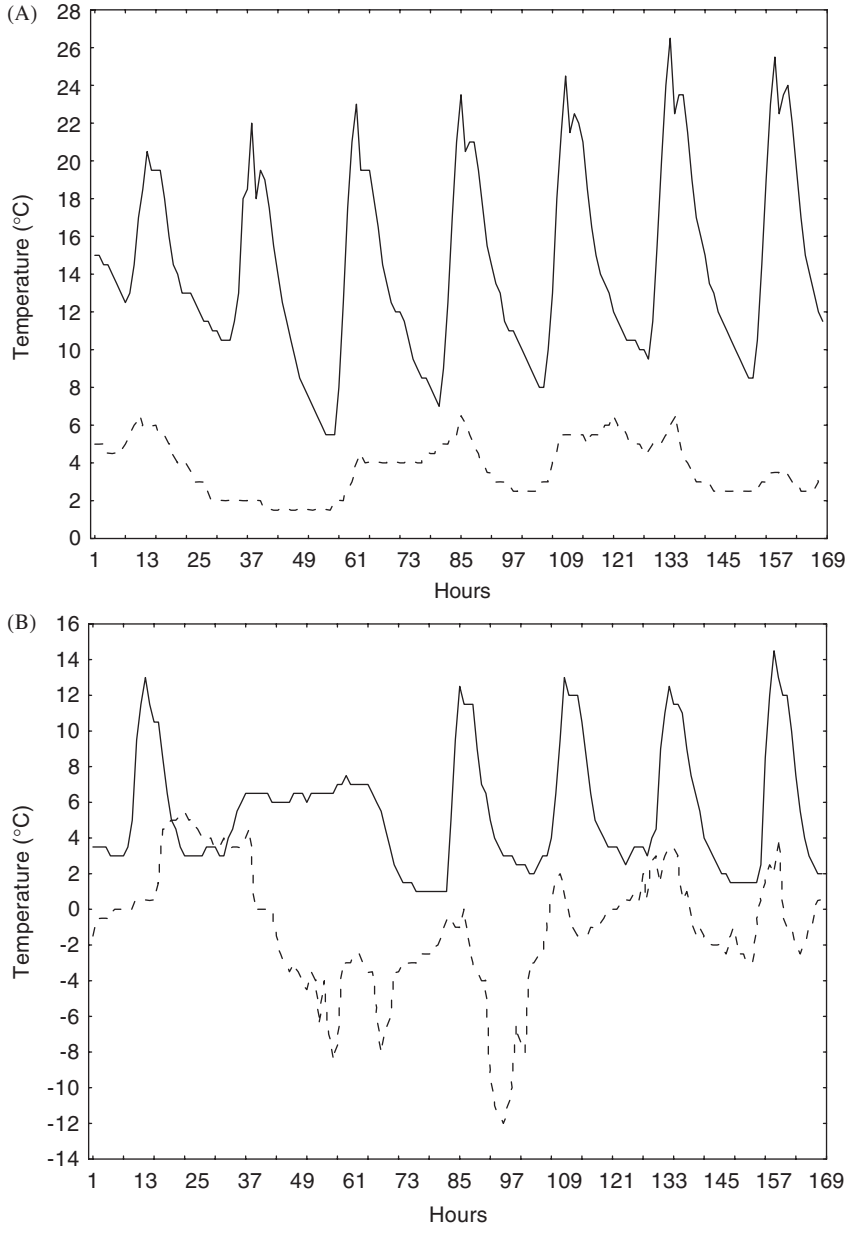



FIG. 4 Hourly temperatures at the soil surface over a week long period in August 2002 for (A) a sea-level site at Lambert's Bay on the west coast of South Africa (solid line) and a sea-level site at sub-Antarctic Marion Island (dashed line), and (B) a site (Sneeukop) at 1960 m above sea level 50 km distant from the Lambert's Bay site (solid line) and at 800 m on Marion Island (dashed line). Note the difference in predictability of temperatures for the Lambert's Bay and Marion Island sites.

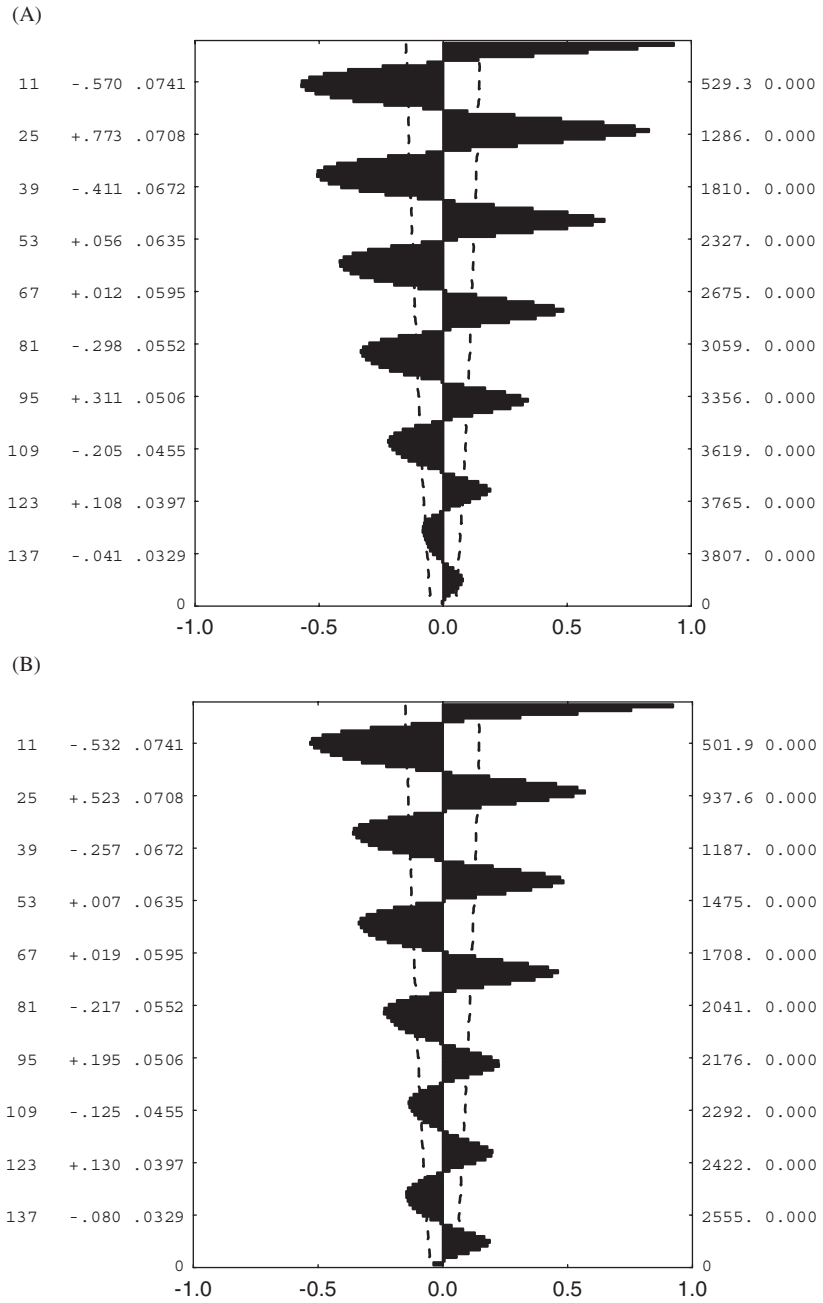
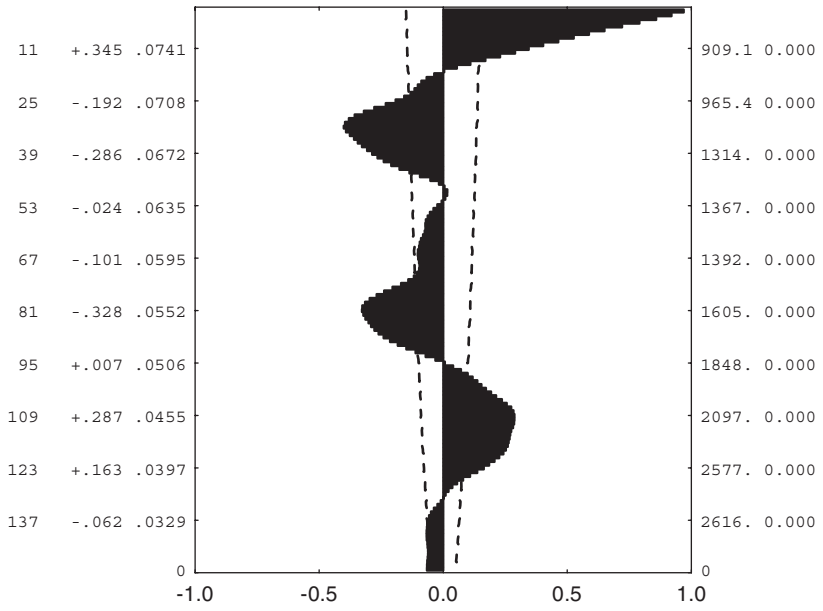



FIG. 5 Autocorrelation plots for hourly temperatures shown in Fig. 4. (A) Lambert's Bay sea-level data, (B) Sneeukop close to Lambert's Bay, (C) Marion Island sea level, (D) Marion Island 800-m site. The dashed lines on each figure represent the 95% confidence intervals, while the values reported to the right of the lags on the *y*-axis are the autocorrelation coefficients and their standard errors.

(C)




(D)

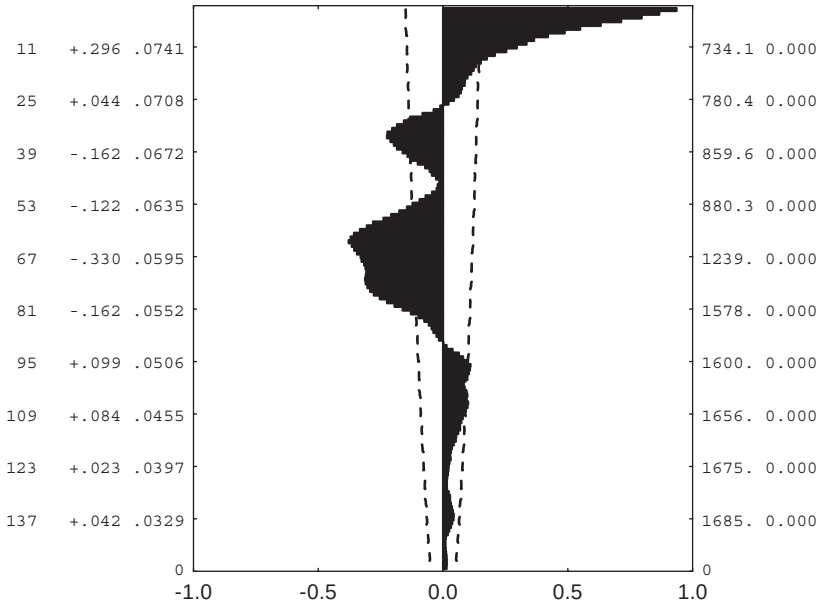



FIG. 5 (continued)

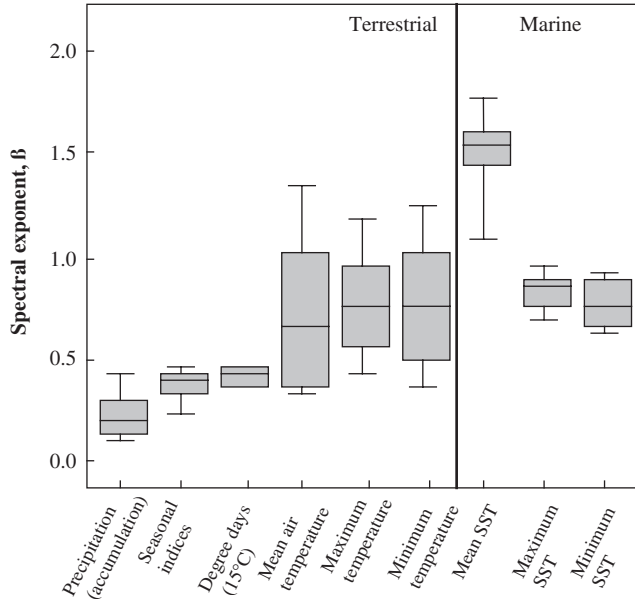



FIG. 6 Box plots of the spectral components for several environmental variables, including sea surface temperature (SST) for terrestrial and marine systems. Lines indicate the median, 75th and 90th percentiles. Redrawn from Vasseur and Yodzis (2004, p. 1149) with permission from the Ecological Society of America.

assessment of long-term variability (with the seasonal component removed), Vasseur and Yodzis (2004) showed that in the case of mean environmental temperature, noise colour varies from mostly white ($0 \leq \beta \leq 0.5$) in terrestrial locations to red–brown (red: $0.5 \leq \beta \leq 1.5$; brown: $1.5 \leq \beta \leq 2$) at coastal locations, to brown for sea-surface temperature data. By contrast, monthly minima and maxima have reddened spectra, whilst precipitation and seasonal indices are characterized by pink noise (Fig. 6). The difference between mostly white spectra at terrestrial locations and reddened noise in marine systems is probably the consequence of the substantial buffering capacity of the sea (Vasseur and Yodzis, 2004). This buffering capacity can probably also explain the mostly white spectra of minimum temperature between 30 and 60° of latitude in the northern hemisphere, and the reddened spectrum in the same areas in the southern hemisphere. Between 30 and 60° N, the land:water proportion is approximately 1:1, whereas between 30 and 60° S, it is 1:15 (Chown *et al.*, 2004a). The absence of a difference in the spectral exponent for maximum air temperature among the hemispheres is readily explained by the fact that absolute maxima differ little among them, whereas variation in absolute minima is much more pronounced (Addo-Bediako *et al.*, 2000; Chown *et al.*, 2004a).

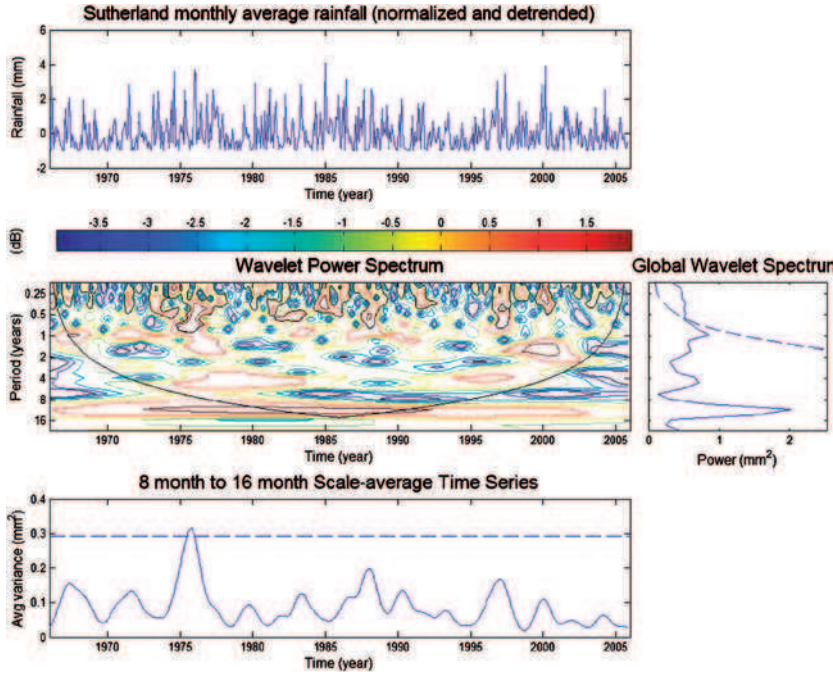



FIG. 7 Wavelet analysis (see Torrence and Compo, 1998) of monthly rainfall data from Sutherland, a high altitude, semi-arid area in the Karoo of South Africa. The upper panel shows the detrended normalized data. The central panel, the wavelet power spectrum with period on the *y*-axis and years on the *x*-axis, and the dark line the cone of influence (with no zero padding), and the global power wavelet spectrum shown to the right thereof. The averaged time series is shown in the lower panel.

If changes in periodic behaviour over a long period take place (i.e. the data lack stationarity), Fourier analyses and assessments of the relationship between spectral density and frequency will not reveal them. Although the scales of variation of the entire series will be apparent, any sequence in these data will remain hidden (Grenfell *et al.*, 2001). Wavelet analysis is a powerful technique that can be used to explore variation in frequency as time progresses by time–frequency analysis of the signal (Fig. 7). Although it is a relatively complex analytical approach, several clear guides to its use are available (e.g. Torrence and Compo, 1998), and it is no longer confined to the geophysical applications in which it has been most popular (e.g. Mélice *et al.*, 2003). Rather, it is being applied to a wide variety of population-level data. For example, wavelet analyses unveiled a substantial change in inter-annual variability of the populations and breeding success of three Antarctic seabird species associated with a shift in environmental conditions (Jenouvrier *et al.*, 2005). Klvana *et al.* (2004) used wavelet

analysis to demonstrate a strong coherence between porcupine feeding scar data and the solar cycle: the first demonstration of a population cycle in mammals that is related to both local climatic fluctuations and the solar cycle. In insect herbivores, a similar relationship was demonstrated at virtually the same time, though using a less complex analytical approach (Selås *et al*., 2004). The populations of several moth species in Norway are inversely correlated with sunspot activity. This seems to be a consequence of enhanced UV-B radiation during low sunspot activity, which requires pigment production by the host plant. Caterpillars prefer leaves exposed to elevated UV-B because the leaves incur metabolic costs producing pigments, so reducing resistance to the herbivores.

Investigations of changes in long-term periodic behaviour are not common in the insect physiological literature. However, it seems likely that they will prove to be useful, especially in understanding long-term changes in insect responses to the environment. These kinds of changes are not unknown in the physiological literature. For example, in the overwintering larvae of *Dendroides canadensis* and *Cucujus clavipes,* initial studies indicated that individuals are freezing tolerant, while investigations in the following years revealed a switch to freeze intolerance (Kukal and Duman, 1989). This shift in cold hardiness strategy is thought to have been a consequence of changes in the thermal environment of the species, though no detailed time-series analyses were undertaken. In the cricket, *Conocephalus discolor*, long-term changes in the environment have resulted in high frequency of an extra-long-winged form in newly established populations of the species, which must have been affected through changes in hormonal regulation of wing production in the species (Thomas *et al*., 2001). The influence of variability and predictability on the evolution of plasticity also means that long-term assessments of the likely stationarity of the environment may provide considerable insight into species responses that might be mediated by plasticity (Stillman, 2003; Helmuth *et al*., 2005).

## 4 Phenotypic plasticity

Although circumstances exist where a specialist will be favoured over a conditional strategist (Berrigan and Scheiner, 2004; van Kleunen and Fischer, 2005), plasticity is optimal under a wide range of conditions (Section 2.2). Appreciation for the commonness of phenotypic plasticity has long existed in the literature on physiological and morphological traits (review in DeWitt and Scheiner, 2004), but it is only relatively recently that its importance in evolution has been realized (West-Eberhard, 1989, 2005). The literature in the field is now substantial, and the idea here is not to review the field, nor to dwell on debates, such as the merits of the character state and polynomial approaches to investigating plasticity, that have long

characterized the field. Recent comprehensive reviews and perspectives provide ready access to this literature, including resolution of several of the debates (e.g. Nylin and Gotthard, 1998; Schlichting and Pigliucci, 1998; Schlichting, 2002; Pigliucci and Murren, 2003; West-Eberhard, 2003; DeWitt and Scheiner, 2004; Pigliucci, 2005). Rather, we focus on several issues that are significant for physiologists concerned with phenotypic plasticity, especially in its more common guises of acclimation or acclimatization. Initially, we dwell briefly on semantic issues, not because we think that creating specific terminology for different forms of plasticity is especially helpful (see West-Eberhard, 2003 for this view and Piersma and Drent, 2003, for a contrary opinion), but because in some cases it is not yet entirely clear how similar or different are the mechanisms underlying responses at different time scales (e.g. Bowler, 2005; Loeschcke and Sørensen, 2005; Sinclair and Roberts, 2005, but see also Chown and Nicolson, 2004, Ch. 5).

### 4.1 TERMINOLOGY

Phenotypic plasticity can be defined as 'the ability of an organism to react to an environmental input with a change in form, state, movement, or rate of activity' (West-Eberhard, 2003). It is often also defined as 'the environmentally sensitive production of alternative phenotypes by given genotypes' (DeWitt and Scheiner, 2004), although in the singular, such a definition could result in neglect of the fact that the initial phenotype of an individual is typically a structure provided by the parent, and therefore is not the product of one genotype (West-Eberhard, 2003; Huestis and Marshall, 2006). The former definition includes all forms of plasticity, and indeed, can be simplified to 'intra-individual variability'. Further, formal, qualification of the term plasticity, and hence a restriction of its definition, has long been used to distinguish between non-adaptive and adaptive responses, active and passive responses, reversible, irreversible and cyclic responses, continuous and discontinuous responses, and those which take place following development, or shorter-term exposures to different environments (see Piersma and Drent, 2003; Bowler, 2005; Seebacher, 2005 for recent examples, and West-Eberhard, 2003, for review of the older literature). By contrast, West-Eberhard (2003) suggested that special terms for these kinds of plasticity are not necessary, but rather that descriptive adjectives should be used to make appropriate distinctions where these are necessary. In a similar vein, DeWitt and Scheiner (2004) argued for broad applicability of the term plasticity, pointing out that the significant issue is the focus on genotype-environment interactions.

These more 'liberal' approaches are well suited to investigations of plasticity in insects. For example, the definitions provided by Piersma and Drent (2003), initially seem appropriate for studies of intra-individual

environmental responses in this group. However, on further consideration it is clear that they are problematic. Thus, 'developmental plasticity' in Piersma and Drent's (2003) sense is not thought to take place within a single individual, whereas this contradicts widely accepted views on plasticity, probably as a consequence of the fact that the distinction between population and individual levels was not explicitly made (see Pigliucci, 2005, Box 1). Likewise, Piersma and Drent (2003) argue that 'developmental plasticity' precludes reversible phenotypic change. However, several recent studies have shown that developmental plasticity in a variety of traits may be either reversible or irreversible. In *Bicyclus anynana*, rearing temperature has a substantial effect on egg size, which is largely reversible by holding adults at different temperatures (Fischer *et al*., 2003, 2006), and in *Lycaena tityrus,* the effects of developmental plasticity on cold shock are similarly reversible in the adult stage (Zeilstra and Fischer, 2005). In the tsetse, *Glossina pallidipes*, developmental plasticity (pupal exposures only) of critical thermal minima and desiccation rate are irreversible following treatments at 29 °C relative to 25 °C, but the pupal treatment was either reversed or had little effect following a 21 °C treatment for these traits, and following both treatments in the case of metabolic rate and critical thermal maximum (Terblanche and Chown, 2006). Similarly, in *D. melanogaster*, mortality induced by cold shock following rearing at a high developmental temperature is little affected by adult acclimation, whereas chill coma recovery time is strongly affected by adult acclimation (Rako and Hoffmann, 2006).

Some confusion has also arisen in the literature as a consequence of distinctions made between responses considered to be genetic ($\approx$ adaptive) and those thought to be non-genetic ($\approx$ plastic). Although widespread, such a distinction is, in DeWitt and Scheiner's (2004) words 'enduring and perennially misleading'. As they point out, plastic responses have a genetic basis, and may be active or passive (see also West-Eberhard, 2003; Fischer *et al*., 2006). Moreover, as has long been clear, genotypes and the environment interact (often simply stated as a G $\times$ E interaction). Unfortunately, the terms plasticity and G $\times$ E interactions are also sometimes confused because of usage of the terms at both the level of individual genotypes and populations of genotypes (Pigliucci, 2005). Because plasticity is defined as the ability of an organism to react to an environmental input, a slope (positive or negative) in the environment–phenotype space indicates plasticity at the individual level, and plasticity at the population level if the average difference among environments across genotypes is considered (Fig. 8). At the population level, statistically significant G $\times$ E interactions refer to the differences in slope of the reaction norms (DeWitt and Scheiner, 2004), whereas at the individual level genotype by environment interactions represent the idea that the genotype and environment interact continuously during an individual's development (Pigliucci, 2005). Much of the insect physiological literature on thermal tolerances and water

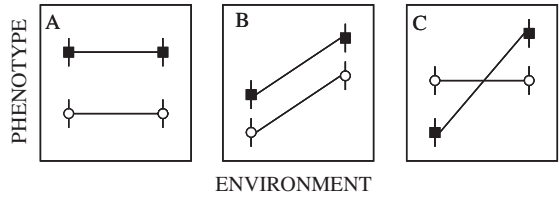



FIG. 8 Reaction norms for two families (circles and squares, mean and standard errors for the phenotypes are shown) demonstrating (A) significant genetic variance, (B) significant genetic and environmental variance, (C) significant genetic, environmental, and genetic by environmental interaction variance. Based on DeWitt and Scheiner (2004, p. 4).

balance is concerned with population-level responses, and discussion of these responses should bear in mind the distinctions between plasticity and G × E interactions made so clearly by DeWitt and Scheiner (2004).

A final potential complication arises when performance curves (see Huey and Kingsolver, 1993; Huey and Berrigan, 1996) or performance functions (Angilletta *et al.*, 2002) and reaction norms (the form a phenotypic response to the environment takes, see Huey and Berrigan, 1996) are equated. There is no reason why a performance curve should not be considered a reaction norm (see Angilletta *et al.*, 2003), and the statistics for analysing aspects of the two are similar in many respects (compare Gilchrist, 1996 and David *et al.*, 1997, see also Kingsolver *et al.*, 2001). However, the complication arises when the response of the performance curves themselves, or components thereof, to various environmental conditions are assessed. Thus, the shape of the performance curve as well as its position, breadth, height, and other components (see Huey and Kingsolver, 1993; Angilletta *et al.*, 2002) might all respond in different ways to environmental conditions imposed during any part of an individual's life (Fig. 9). The form of these responses also constitutes a reaction norm. Arguably, the most appropriate way to deal with such potential complications is to be explicit about what the subject is of the work. Where variation in performance curves is being assessed, use of the term 'reaction norm' should be restricted to the response of the curves, rather than being meant to imply the curves too.

### 4.2 ACCLIMATION AS A FORM OF PLASTICITY

With the exception of metabolic scaling, few topics in evolutionary physiology have generated as much recent, vigorous discussion as has acclimation and whether or not it is beneficial (reviews in Huey *et al.*, 1999; Angilletta *et al.*, 2006). Acclimation (in the laboratory) and acclimatization (in the field) are both terms coined to describe intra-individual variability. Therefore, they describe forms of phenotypic plasticity (see Huey and

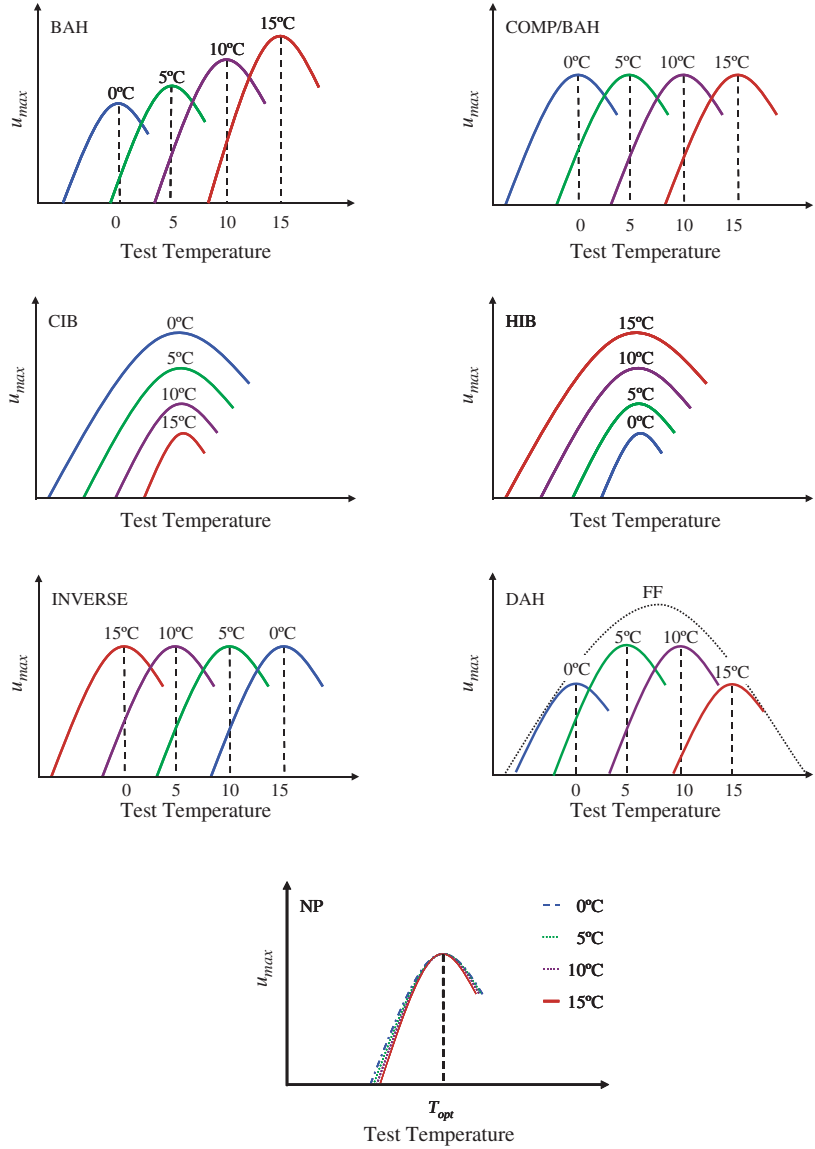



FIG. 9 Predictions from each of the major hypotheses for the response of individual performance curves to acclimation. In each case four acclimation temperatures from low to high are indicated (blue (0 °C), green (5 °C), purple (10 °C), red (15 °C)), and in one case the expectation for field fresh (FF) individuals is also shown. BAH = beneficial acclimation hypothesis, COMP/BAH = complete temperature compensation (an instance of BAH), CIB = colder is better, HIB = hotter is better, IAH = inverse acclimation hypothesis, DAH = deleterious acclimation hypothesis, NP = no plasticity. Redrawn from Deere and Chown (2006).

Berrigan, 1996; Huey *et al*., 1999). Physiologists have long held the view that phenotypic change by an individual in advance of, or in response to, a changing environment is beneficial (see Prosser, 1986; Cossins and Bowler, 1987; Hochachka and Somero, 2002 for access to the literature, and Shreve *et al*., 2004, for a recent example). This view has been recast as the beneficial acclimation hypothesis (BAH), defined by Leroi *et al*. (1994) as 'acclimation to a particular environment gives an organism a performance advantage in that environment over another organism that has not had the opportunity to acclimate to that particular environment'. While it does not explicitly cover responses in anticipation of an environmental change, this definition has been used by many recent investigations as a departure point for examining the extent to which acclimation can be considered beneficial (see Huey *et al*., 1999 for review). In addition, the majority of these studies have included explicit *a priori* alternatives, a strong inference approach that was typically lacking from the previous physiological literature (Huey and Berrigan, 1996). Most of these more recent studies have found little support for the beneficial acclimation hypothesis (Leroi *et al*., 1994; Zamudio *et al*., 1995; Bennet and Lenski, 1997; Sibly *et al*., 1997; Woods, 1999; Gibert *et al*., 2001; Gilchrist and Huey, 2001; Woods and Harrison, 2001; Stillwell and Fox, 2005). Rather, in each case one or more of the alternative hypotheses (Fig. 9) could not be rejected.

Considering the wide range of scenarios under which plasticity is likely to be favoured (Section 2.2; also Scheiner, 1993; Agrawal, 2001), this lack of support for the BAH is counter intuitive. However, as is clear from Section 2.2, several circumstances exist in which acclimation is unlikely to be beneficial. Moreover, under some conditions, such as if lag times are substantial, plasticity might not readily evolve. Wilson and Franklin (2002) argued that the majority of thermal acclimation tests of the BAH are neither direct nor complete because they assess the adaptive significance of 'developmental plasticity', rather than investigating what comparative physiologists regard as acclimation (or acclimatization) (see Spicer and Gaston, 1999, pp. 32–38; Willmer *et al*., 1999, pp. 9–12). That is, many past assessments of phenotypic plasticity have involved alteration of rearing regimes and subsequent assessment of adults (which implies substantial lag times), rather than assessment of phenotypic alterations within a given life stage. Thus, in Wilson and Franklin's (2002) view, these tests are confounded by the fact that several different kinds of plasticity are being assessed simultaneously. It has also been suggested that some of the alternative hypotheses are not mutually exclusive and that it is, in consequence, difficult to design experiments to distinguish between them (Angilletta *et al*., 2006). Finally, stressful environmental treatments might have compromised tests of the BAH by impairing organismal performance (Wilson and Franklin, 2002; Woods and Harrison, 2002), and a focus on the entire suite of characters that constitute fitness is likewise problematic (Woods and Harrison, 2002).

Several proposals have been made to resolve what appears to be a hung jury on the question of beneficial acclimation. These include adopting a strong inference approach and selecting environmental conditions with care to ensure that the effects of stressful conditions are fully assessed (and perhaps using independent measures of stress such as the presence of heat shock proteins) (see discussion in Hoffmann, 1995; Hoffmann and Hewa-Kapuge, 2000; Loeschcke and Hoffmann, 2002; Wilson and Franklin, 2002; Woods and Harrison, 2002). Careful consideration of the alternative hypotheses in the context of appropriate statistical methods (e.g. orthogonal polynomial contrasts in ANOVA – Huey *et al*., 1999) should also alleviate problems associated with hypothesis testing. For example, Angilletta *et al*. (2006) suggested that the 'colder is better' and 'developmental buffering' hypotheses are not mutually exclusive because the former posits increased body size at low temperatures whereas the latter is based on a size-independent mechanism. However, as Huey *et al*. (1999) made clear, 'colder is better' also suggests that performance could be enhanced following low-temperature treatments by mechanisms not associated with size. Therefore, the two hypotheses could be mutually exclusive (see Fig. 9). Explicitly assessing different forms of plasticity (e.g. hardening, acclimation within a life stage, and developmental plasticity) can also provide a fresh perspective on the question. For example, exposure of *Drosophila melanogaster* to low-temperature treatments for brief periods of a few hours (hardening), two days (acclimation), and for two generations (developmental plasticity) revealed substantial complexity in fly responses, some of which could be considered beneficial (Rako and Hoffmann, 2006; see also Nielsen *et al*., 2005). Broader application of these approaches is essential if the significance of phenotypic plasticity for the evolution of physiological traits, and for changes in the distribution and abundance of organisms are to be more fully comprehended (Section 2.4 ; Sultan, 2004; Dybdahl and Kane, 2005).

### 4.3 'UNINTENTIONAL' ACCLIMATION

Any population exposed to a novel environment is expected, at least in the longer term, to adapt to that environment, or at the very least respond to selection imposed by that environment. Reponses to selection are indeed common both in the laboratory and in the field (e.g. Huey *et al*., 1991; Gibbs, 1999; Hoekstra *et al*., 2001; Kingsolver *et al*., 2001). One unintended consequence of this response is that organisms held in the laboratory for several generations adapt to the laboratory conditions (Harshman and Hoffmann, 2000; Matos *et al*., 2000; Sgrò and Partridge, 2000). Differences between laboratory colonies and field populations have been documented for many traits and species, including cold and heat tolerance in flies (Zatsepina *et al*., 2001), antennal sensilla chemo- and

mechanoreceptors in Hemiptera (Catala *et al*., 2004), pheromone communication between sexes in the screwworm *Cochliomyia hominivorax* (Hammack, 1991), and $CO_2$ anaesthesia effects on knockdown and recovery times in cockroaches (Branscome *et al*., 2005). Such laboratory adaptation can also take the form of a relatively rapid decline in stress resistance. For example, in *Drosophila melanogaster*, starvation and desiccation resistance declined from LT50 values of 50.1–35.9 h, and 14.3–8.9 h, respectively over a period of four years (Hoffmann *et al*., 2001). However, not all traits respond so strongly to long-term laboratory culture (Krebs *et al*., 2001).

Therefore, rapid responses to selection often seen in the laboratory might represent the reacquisition of responses to more stressful conditions experienced by the population before it was taken into culture. The accumulation of mutations in culture, which can have significant effects on responses to laboratory selection, also appears to be pervasive (Harshman and Hoffmann, 2000). In consequence, investigations using laboratory selection, which provides a useful and essential complement to comparative studies (Kingsolver and Huey, 1998; Gibbs, 1999; Feder and Mitchell-Olds, 2003), must take due cognisance of laboratory adaptation.

In a similar fashion, holding organisms for substantial periods in the laboratory could give rise to substantial, unintended, acclimation effects. It is widely appreciated that insects can respond rapidly to a given environmental treatment and to its relaxation (e.g. Lee *et al*., 1987a; Hoffmann *et al*., 2003b; Chown and Nicolson, 2004; Rako and Hoffmann, 2006; Terblanche *et al*., 2006). Such laboratory responses form the basis of a large and proliferating physiological field aimed at investigating the nature, time course, and mechanistic underpinnings of phenotypic plasticity. What is perhaps less widely acknowledged is that unintended acclimation can confound investigations (though see Spicer and Gaston, 1999). One recent demonstration of the significance of this problem is provided by an investigation of the scaling of avian metabolic rate (McKechnie *et al*., 2006). Captive birds have a shallower metabolic rate–body mass relationship than wild birds because small birds tend to upregulate basal metabolic rate in captivity, while the converse is true in large birds. The same kinds of responses could confound physiological investigations in arthropods. In the whip-spider, *Damon annulatipes*, mean metabolic rate declined substantially, from 30.2 to 21.8 $\mu l\ CO_2\ h^{-1}$, despite no change in mean body mass, following two weeks in the laboratory (Terblanche *et al*., 2004). The same trend has been found in the scorpion *Uroplectes carinatus* (Fig. 10). These declines in metabolic rate are likely a consequence of reduced temperature variation, less demanding foraging requirements, and absence of the need to avoid predators (Hoffmann *et al*., 2001; Terblanche *et al*., 2004). Simple simulations illustrate that, if these kinds of effects are more

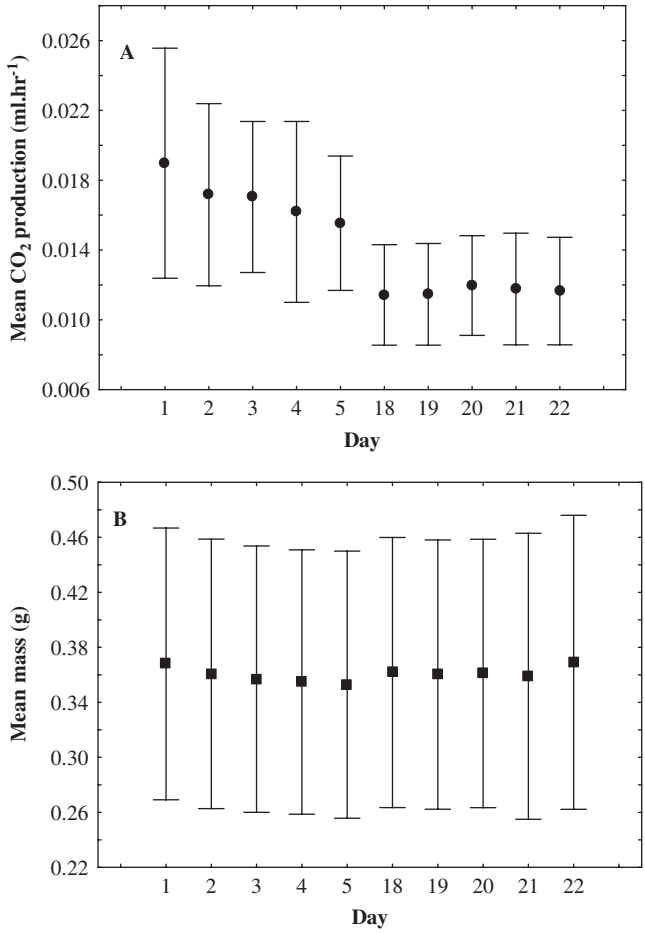



FIG. 10 A rapid decline is found in whole-animal metabolic rate (A) but not in body mass (B) with introduction to stable laboratory conditions in the scorpion *Uroplectes carinatus*. Mean standard metabolic rate ($CO_2$ ml $h^{-1} \pm 95\%$ confidence intervals) recorded using flow-through respirometry at 25 °C and body mass (in g) from each trial day during acclimation to constant conditions (25 °C) in the laboratory.

common than has been assumed, they will have to be taken into consideration in future, especially, comparative studies. Assume that metabolic rate scales as mass$^{0.70}$, with little variation as a consequence of different life histories – a simplistic assumption (Kozłowski *et al.*, 2003; Brown *et al.*, 2004; Clarke, 2004), but one useful for present purposes. If metabolic rate declines with an exponential decay function ($y = \mathrm{MR}\ e^{-0.15t}$, where $t$ = hypothetical time in the laboratory), and the amount of time spent in the laboratory varies at random among the species (or individuals/populations)

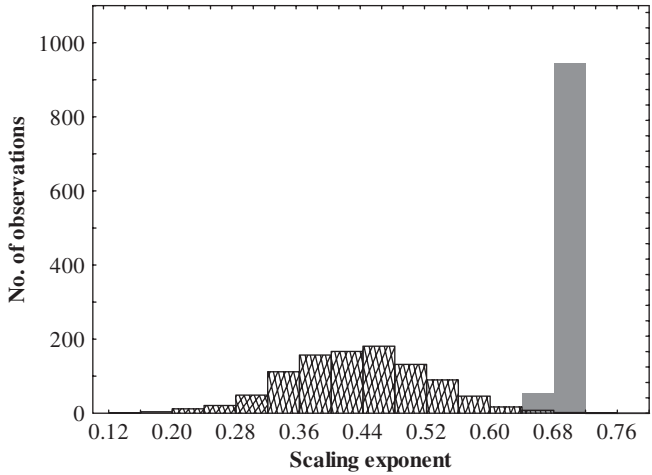



FIG. 11 Variation induced by laboratory acclimation around a hypothetical metabolic rate-body mass scaling relationship. The solid bars represent a hypothetical metabolic rate-body mass scaling relationship for animals ($n = 50$ individuals, using 1000 random numbers re-sampled with replacement using Microsoft Excel) that are all in the same acclimation state (i.e. only field collected). An exponential decay function ($y = \mathrm{MR}\ e^{-0.15t}$ ,where $t$ = hypothetical time in the laboratory) was applied to these data to simulate a possible acclimation-induced decline in metabolic rate and how this may affect the scaling exponents (hatched bars). A random series of time intervals were generated (ranging from 0 to 5) and applied to metabolic rate data using the exponential decay function.

of interest, then the form of the relationship can change substantially (Fig. 11). Most notably, there is considerable increase in the variation of slope coefficients.

What unintended laboratory acclimation means for past comparative investigations is not obvious, although the nature of the question and the likely signal-to-noise ratio of the study will determine the importance of the unintended effects (see discussion in Chown *et al.*, 2003; Hodkinson, 2003). Nonetheless, it is clear that comparison of individuals freshly retrieved from the field with those held in the laboratory, bearing in mind that seasonal acclimatization is also common (Chown *et al.*, 2003; Chown and Nicolson, 2004), could go some way to resolving these issues. Similarly, field-cage experiments (such as reciprocal transplants) (e.g. Jenkins and Hoffmann, 1999; Hoffmann *et al.*, 2003c) may be revealing. However, it should not be forgotten that in many instances the very subject of investigation is phenotypic change in response to manipulation of one or more environmental variables while all others are held constant. In this case, laboratory treatments and investigations of individual responses are the only way to proceed, but their unintentional consequences should not be neglected.

## 5 Sensing

Any response to the environment, whether it is a conditional response, or one that eventually becomes fixed, requires a sensing mechanism or receptor (Denlinger *et al*., 2001; Danks, 2003). Lag times, unpredictability, and inscrutability of the environment are widely discussed in the literature on the evolution of phenotypic plasticity, as are the nature and time course of, and mechanisms underlying organismal responses. The perception of the environment dictates the speed of response to change (see also Robertson, 2004a). Therefore, knowledge of the mechanisms that underlie perception is important for determining the way in which the animal is likely to perceive and respond to a changing environment. Accurate environmental perception enables insects to take advantage of optimal conditions, ultimately contributing to the animal's success in a particular environment. The relative timing and reliability of cues not only has behavioural implications, but also has both physiological and ecological consequences, ranging from the preparation for and response to diurnal and seasonal physiological changes, to physiological reorganization during dormancy, and the likelihood that phenotypic plasticity will evolve. Nonetheless, in the context of plasticity, sensing mechanisms (or receptors) have typically received much less attention than other physiological traits. Consequently, this is a fertile field for investigation, though it presupposes that much of the basic information on sensing is available (see Chown and Storey, 2006 for an analogous discussion). As we show in this section, progress in modern understanding of temperature and moisture (hygro-) sensing differs appreciably, and has some way to go before it can be readily integrated into investigations of whole-animal physiological responses to the environment.

### 5.1 Detecting Changes in external environmental temperature

For at least the past century, it has been clear that insects are capable of sensing and responding to temperature variation (reviewed in Blum, 1985; Chown and Nicolson, 2004), as is attested by studies of body temperature regulation. For example, behavioural thermoregulation in a temperature gradient has been shown in a wide variety of species, including cockroaches (Murphy and Heath, 1983), grasshoppers (Lactin and Johnson, 1996; Forsman *et al*., 2002), bugs (Lazzari, 1991; Guarneri *et al*., 2003; Minoli and Lazari, 2003; Schilman and Lazzari, 2004), moths (Kuhrt *et al*., 2006), beetles (Roberts *et al*., 1991; Ybarrondo, 1995; Jian *et al*., 2002), flies (Huyton and Brady, 1975; Yamamoto, 1994), and ants (Roces and Nunez, 1995). It is largely assumed that body temperature preferences evolve (see Garland *et al*., 1991; Angilletta *et al*., 2006), although only a few demonstrations exist. Thus, it is not clear to what degree natural selection has

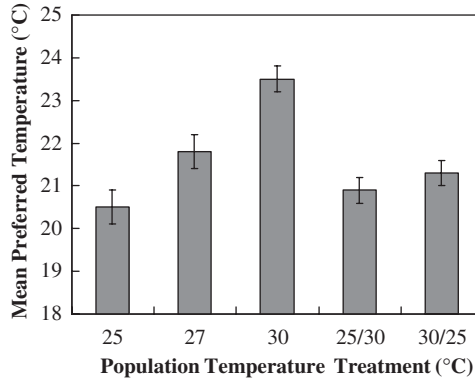



FIG. 12 Mean preferred temperatures (± SE) in *Drosophila melanogaster*. Values represent tenth generation 25, 27, and 30°C-reared populations and reverse temperature treatment populations (i.e. reversible plastic component), 25/30 °C and 30/25 °C (females only). The 25, 27, and 30 °C groups are significantly different, while the 25/30 °C and 30/25 °C do not differ (although both of the latter reverse treatments differ from the 30 °C group). Figure redrawn from Good (1993) with permission from Elsevier.

been responsible for the origin and maintenance of preferred body temperature variation among insect species. However, a positive response to laboratory selection has been observed in *Drosophila melanogaster* (Good, 1993). After 10 generations, flies reared at warmer temperatures showed an increase in preferred body temperature (Fig. 12). Partial reversal of the shift in preferred body temperature provided evidence for an environmental component to the change (Good, 1993; see also Murphy, 1986; Forsman *et al*., 2002). These findings also suggest that geographic clines in preferred body temperature should not be uncommon, and this seems to be the case in some species (e.g. the grasshoppers *Melanoplus sanguinipes* and *Xanthippus corallipes*) (Ashby, 1997; Rourke, 2000; Samietz *et al*., 2005), but not others (e.g. *Drosophila immigrans* and *D. virilis*, Yamamoto, 1994).

Other factors may also play a crucial role in determining temperature preference. Remarkably, individuals of the nematode *Caenorhabditis elegans* select temperatures at which they were reared, while specifically avoiding temperatures at which they were starved (Mori, 1999). Thus, while temperature optimization may be critical in determining organism survival, more immediate fitness consequences may force animals to use behavioural means to override physiological function under certain circumstances (Huey *et al*., 2003). However, such a mechanism has not yet been demonstrated in insects (Forsman *et al*., 2002).

The proximate mechanisms for sensing environmental temperature are specialized sensillae, which may be found on various body parts in terrestrial arthropods (see Must *et al*., 2006), and in particular on the

antennae in insects. Central temperature receptors, which measure body temperature, for example, in the prothoracic ganglion of the cockroach, also exist (Murphy and Heath, 1983; Janiszewski, 1986).

Much evidence for temperature sensing has been provided by electrophysiological studies, used effectively to show that temperature causes depolarization of specific antennal cells (reviewed in Altner and Loftus, 1985; Merivee *et al*., 2003; Must *et al*., 2006), which, in the case of *Drosophila*, are usually located in the third antennal segment (e.g. Shanbhag *et al*., 1995; Sayeed and Benzer, 1996). These peripheral temperature receptors increase their firing rate as the temperature is lowered (in the case of cold receptors; e.g. Loftus, 1968; Nishikawa *et al*., 1992; Merivee *et al*., 2003) or raised (in heat receptors in vertebrates, see Patapoutian *et al*., 2003), but display little electrophysiological activity in response to baseline temperature within the normal range (Nishikawa *et al*., 1992). Consequently, thermosensory neurons respond in a phasic-tonic manner to rapid temperature changes, and several cold cell responses can occur (including changes in peak frequency and action potential (firing) rate). However, the specific characteristics of both the phasic and tonic changes can vary substantially among cells within sensilla, individuals, and species (Merivee *et al*., 2003; Must *et al*., 2006).

In temperature sensitive neurons, the receptor cells probably do not function as a simple thermometer. Rather, neuronal firing rates are also influenced by the direction and rate of temperature change (e.g. Nishikawa *et al*., 1992; and reviewed in Patapoutian *et al*., 2003; Must *et al*., 2006). Thus, the relationship between temperature sensing at the neuron and temperature perception in the central nervous system (CNS) is complex because different cells respond in different ways to variation in temperature (Nishikawa *et al*., 1992; and see Merivee *et al*., 2003). Additional complexity arises because different cooling rates elicit different neuronal firing rates among the various receptor types (Nishikawa *et al*., 1992). The magnitude of temperature change in, for example, step-wise changes can also influence the steady-state firing rates (Nishikawa *et al*., 1992; Ehn and Tichy, 1994), and considerable temporal changes in firing rates, often with rapid phasic (i.e. transient) changes occurring during the first few seconds of a response, have also been found (e.g. Ehn and Tichy, 1994; Must *et al*., 2006). A variety of temperature response types for neuronal firing rates can be distinguished in terrestrial arthropods, and are discussed in detail by Must *et al*. (2006).

In the case of steady-state changes in temperature, for most insects investigated to date, cold cells provide information on warmer temperatures via a reduction in neuronal firing rates (Must *et al*., 2006; see also Nishikawa *et al*., 1992; Merivee *et al*., 2003). During rapid temperature changes, warming results in a long inter-spike period followed by a similar reduction in nerve impulse activity (Merivee *et al*., 2003). Currently,

however, there is relatively little evidence in insects for heat receptors which respond with an increase in firing rates to warmer, stable temperatures (but see Must *et al.*, 2006). During cooling to a new cold temperature, the phasic component indicates temperature decrease at the start of a temperature change, while the tonic component decreases more slowly (depending on the magnitude of the temperature change) and stabilizes at the new level of constant temperature. Thus, a considerable amount of information is transmitted to the CNS regarding the insect's immediate thermal environment, although how this information is processed and integrated remains unclear at present (Merivee *et al.*, 2003).

Body temperature preference typically represents a value well within the range of temperatures experienced in an organism's natural environment, and variation in precision, and thus possibly sensitivity, of temperature regulation on a thermal gradient is not uncommon (Yamamoto, 1994; Sayeed and Benzer, 1996; see also Murphy, 1986). Consequently, different levels of temperature sensitivity (i.e. variation in neuronal responses to temperature stimuli) among insect species are unsurprising (discussed in Nishikawa *et al.*, 1992), and some evidence exists for inter-specific variation in the ability to discriminate temperature changes (i.e. resolution). For example, sensory neurons in *Drosophila* larvae can detect temperature variation at a resolution of $<1\,^{\circ}C$ (Nishikawa *et al.*, 1992; Liu *et al.*, 2003), while *Speophyes lucidulus* is conservatively estimated to be capable of resolving temperature changes of $0.7\,^{\circ}C$ (Altner and Loftus, 1985; see also Hess and Loftus, 1984). The ground beetle, *Pterostichus aethiops*, shows changes in firing rates of campaniform sensilla induced by temperature changes of as little as $0.1\,^{\circ}C$ (Merivee *et al.*, 2003). By contrast, a single warm cell from the spider *Cupiennius salei* can resolve differences in warming to $0.4\,^{\circ}C$ (Ehn and Tichy, 1994), while a tropical tick's warm cell can resolve temperature to $0.6\,^{\circ}C$ (Hess and Loftus, 1984).

In insects, the cellular mechanisms of temperature sensation have received less attention than, for example, in vertebrates (Clapham, 2003; Liu *et al.*, 2003; Patapouitian *et al.*, 2003). Nonetheless, despite the underlying differences in thermal biology of ectothermic invertebrates and endothermic vertebrates, it appears that their thermal sensory mechanisms may be conserved at the molecular level (Liu *et al.*, 2003; Patapouitian *et al.*, 2003). Accumulated evidence suggests that the primary temperature sensors in the sensory nerve endings of mammals belong to the temperature receptor protein superfamily of cation channels and that these proteins underlie the cellular processes that result in nerve depolarization (Voets *et al.*, 2004). At the cellular level, only recently has it been shown that the invertebrate temperature-activated transient receptor potential ion channel (thermoTRP) families found in *Drosophila* and the nematode worm, *Caenorhabditis elegans,* can be directly activated by temperature (Viswanath *et al.*, 2003) although there is some variation among the

vertebrate and invertebrate TRP systems. Specifically, Viswanath *et al.* (2003) showed that the *Drosophila* orthologue of the mammalian cold-activated ion channel ANKTM1 responds to warming rather than cooling. Therefore, while the thermosensing function may be well conserved from an evolutionary perspective (i.e. the proteins themselves are present in both vertebrate and invertebrate organisms; see also Rosenzweig *et al.*, 2005) a large degree of flexibility in the TRP responses to temperature can be found (Viswanath *et al.*, 2003).

Typically, TRPs are identified by their homology rather than by ligand function, and can serve multiple purposes, many of which are not necessarily related to temperature sensation (Clapham, 2003). Several different mechanisms have been proposed for how the TRPs act as ion gates (reviewed in Clapham, 2003 and Voets *et al.*, 2004 for thermoTRPs). Temperature variation could result in production and binding of ligands that activate channels. By contrast, the channel proteins could undergo some form of temperature-dependent structural changes, thereby resulting in channel opening. Finally, changes in membrane tension, facilitated by lipid bilayer re-arrangements, may cause temperature-dependent activation of thermoTRPs (Clapham, 2003). In mammalian cells, Voets *et al.* (2004) found that temperature sensitivity is regulated by the trans-membrane voltage and ambient temperature variation results in graded shifts in the voltage dependence of channel activation.

Marked variation in the expression and temperature sensitivity of thermoTRPs exists, hence they are grouped into distinct types of sensory neurons according to function. For insects, two key families, with several forms of thermoTRPs in each family, are described: the melastatin family (containing e.g. TRPM8 and ANKTM1), and the vanilloid family (containing TRPV1-4 and TRPA1). In mammals, at least six families are recognized (Clapham, 2003). It is not clear from the available literature if these other thermoTRP families are important, or whether they are present at all, in insects. It has been suggested that TRPA1 may play an important role in thermotaxis in *Drosophila* (Rosenzweig *et al.*, 2005). TRPA1 knockout (using RNA interference) eliminates the avoidance of high temperatures in a thermal gradient, and the expression of this family of ion channel protein occurs in cells not previously thought to have a function in thermosensation (Rosenzweig *et al.*, 2005). (It is worth noting that similar results occur in peripheral temperature receptor ablation experiments in cockroaches, i.e. high temperatures are no longer avoided; Murphy (1986)). For example, Rosenzweig *et al.*, (2005) found some evidence for TRP1A expression in two pairs of cells adjacent to the mouthhooks and in the developing gut. Generally, however, receptors in the melastatin family respond to temperatures in the 17–25 °C range, while the vanilloid receptors are sensitive across the 33–52 °C range. Currently, there is little information available documenting how, if at all, thermoTRP family

composition in sensilla may vary among insect taxa or within species (e.g. along geographic clines).

Five recent findings in temperature sensing strike us as being important from an evolutionary and ecological physiology perspective. First, the perception of temperature can interact with mechanical, electrical (Godde and Haug, 1990), and hygric stimuli (Nishikawa *et al*., 1992; Inoshita and Tanimura, 2006) to alter the neuronal signal (firing rate) (and see Voets *et al*., 2004). Second, temperature (both heat and cold) activation of thermoTRPs can occur in cell-free areas, thereby suggesting that temperature-dependent binding of second messengers is not an important process in the activation of TRPs (Voets *et al*., 2004). This is important because it markedly distinguishes thermoTRPs from classical ion-channels. Third, temperature sensitivity is at least partially dependent on the transmembrane voltage and not solely on temperature, and therefore this voltage can contribute to the fine-tuning of cold and heat sensitivity in sensory cells (Voets et al., 2004). Fourth, in insects, temperature sensation can occur in cells located outside of the antennae (see e.g. Sayeed and Benzer, 1996; Liu *et al*., 2003), and more specifically, in cells not previously thought to have thermosensory functions (e.g. Rosenzweig *et al*., 2005). Furthermore, it has also been suggested that thermoTRPs can sense intra- and extracellular temperature variation (Clapham, 2003). Finally, it has been demonstrated that circadian clock proteins (FRQ) in the yeast, *Neurospora crassa,* could be regulated by thermosensitive gene splicing (at the gene-translation level) and may play a crucial role in temperature sensing (Diernfellner *et al*., 2005), although the importance of such a mechanism requires confirmation in insects.

### 5.2 DETECTING CHANGES IN WATER AVAILABILITY

For reasons similar to those outlined in the previous section, it is apparent that insects can detect changes in external moisture conditions. These include the presence of hygrosensors located on the antennal arista in flies (e.g. Rees, 1970; Sayeed and Benzer, 1996) and antennae of cockroaches (Yokohari, 1978; Tichy, 2003), the demonstration of hygropreference in a humidity gradient (e.g. Hayward *et al*., 2000, 2001; Steidle and Reinhard, 2003; Walters and Mackay, 2003), and electrophysiological studies showing changes in nerve impulse frequency with altered ambient humidity (e.g. Yokohari, 1978; Tichy, 2003). However, the mechanisms of hygrosensing in insects are less clearly elucidated than those of thermosensing, probably because of the perceived intractability of the approaches required for its investigation (though see Tichy, 2003), despite its importance (Edney, 1977; Hadley, 1994; Tauber *et al*., 1998; Chown and Nicolson, 2004). Regardless of the reasons, it is clear that knowledge of thermosensory mechanisms in insects, especially at the cellular level, is more advanced than that

of hygrosensory mechanisms. Consequently, in this section we also draw on information from non-insect arthropod taxa (e.g. Collembola).

That terrestrial arthropods should have particular hygropreferences seems a reasonable proposition, although this has not been demonstrated frequently in insects. Where hygropreference is demonstrated, this is typically done for pests of stored products (e.g. Jian *et al*., 2005) and their potential control agents (e.g. Steidle and Reinhard, 2003), or for vectors of diseases (e.g. Lorenzo and Lazzari, 1999). Non-random hygropreferences have often been demonstrated by means of only two humidity options (either high or low) (e.g. Prange and Hamilton, 1992; Jian *et al*., 2005) and more seldom using a range of humidities, as is usually the case in temperature gradient experiments (but see e.g. Roces and Kleineidam, 2000; Hayward *et al*., 2001; Walters and Mackay, 2003). Regardless, ecological evidence suggesting variation in species' ambient moisture preferences is seldom linked with hygropreference in a gradient (but see Hayward *et al*., 2004b). Specifically for insects, clear humidity preferences have been demonstrated in ants (North, 1991; Walters and Mackay, 2003; but see Roces and Kleineidam, 2000), bugs (Roca and Lazzari, 1994; Lorenzo and Lazzari, 1999; Guarneri *et al*., 2003), beetles (Weston and Hoffman, 1991; Weissling and Giblindav, 1993), and wasps (Steidle and Reinhard, 2003).

In some terrestrial arthropods, no clear hygropreference has been found. For example, the ant, *Atta sexdens rubropilosa* (Roces and Kleineidam, 2000), and the mite, *Lauroppia translamellata* (Hayward *et al*., 2000), do not show distinct humidity preferences. However, hygropreference may be influenced by the physiological state (e.g. desiccation) and ambient temperature (see e.g. Jones, 1950; Hayward *et al*., 2001), or even photoperiod (e.g. North, 1991) experienced by individuals. Therefore, demonstrations of a lack of hygropreference need to consider hydration state before concluding a lack of behavioural hygroregulation exists in a species. For example, in mites (Jones, 1950) and ticks (Lees, 1948), prior desiccation resulted in higher preferred humidity levels. Evidence also exists for a preference in *Cryptopygus antarcticus* for higher humidity levels at elevated temperatures (Hayward *et al*., 2001) (Fig. 13), possibly reflecting a similar physiological process to the former example. An alternative argument, however, may be that species with higher desiccation resistance do not require careful hygropreference (discussed in Hayward *et al*., 2000, 2004b). When faced with a high and low humidity option, at higher temperatures ($>40\,^{\circ}C$) grasshoppers prefer low humidity, possibly to facilitate evaporative cooling (Prange and Hamilton, 1992). In these examples, understanding the cellular-level mechanism of hygrosensing and comparing these responses with desiccation, resistance/tolerance could shed light on the underlying mechanisms.

Electrophysiological studies have confirmed the presence of hygroreceptors on, among others, the antennae of caterpillars, bees, mosquitoes, locusts, bugs, flies, stick insects, and cockroaches (see Altner and Prillinger,

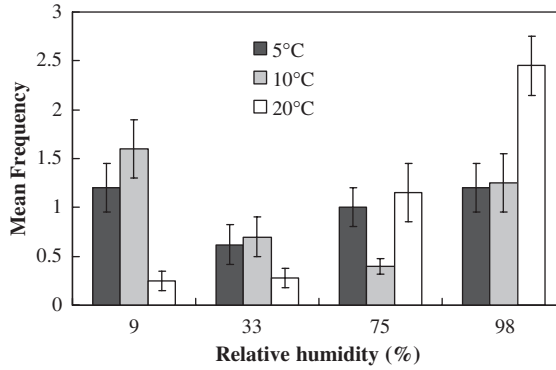



FIG. 13 Mean frequencies (± SE) indicating the distribution of *Cryptopygus antarcticus* within a linear humidity gradient at 5, 10, and 20 °C. These data show that at higher temperatures *C. antarcticus* prefers higher relative humidity. Figure redrawn from Hayward *et al.* (2001) with permission from Elsevier.

1980; Tichy and Loftus, 1996; Tichy, 2003). For example, *Periplaneta americana* has hygroreceptors that increase neuron impulse frequency in response to higher humidity (moist receptors) and dry receptors that increase impulse frequency in response to lowered humidity (see e.g. Waldow, 1970; Yokahari, 1978; Tichy, 2003). Both the moist and dry receptors can be present in the same sensillum, and it has been suggested that the integration of their signals in the CNS may be important for functional responses to altered ambient moisture (reviewed in Altner and Prillinger, 1980). Only recently, however, has it been possible to demonstrate that impulse frequencies of moist and dry receptors are also sensitive to the rate of change in relative humidity (Tichy, 2003). Typically, previous electrophysiological experiments focused on step-wise changes rather than graded responses. High neuron impulse frequencies of the moist cells signal high humidity and *vice versa*. However, at a given humidity level, the response frequency is even higher when the humidity continues rising. The hygroreceptors are most sensitive to low rather than high rates of humidity change. These results therefore suggest continuous input about the state of the moisture in the ambient air, and may provide an early warning of changing humidity conditions (Tichy, 2003). Some evidence also exists for inter-specific variation in hygrosensing abilities. The spider, *Cupiennius salei*, is capable of discriminating between relative humidities differing by 10% (Ehn and Tichy, 1994), while perception of humidity change in *Periplaneta* is approximately double the resolution, with responses to humidity in the order of 5% at the CNS (Nishino *et al.*, 2003).

Within the context of humidity transduction, the structure and function of insect hygroreceptors have been thoroughly reviewed by Tichy and Loftus (1996). Several models have been proposed to explain the humidity

transduction process, but three of these seem to be the most likely (Tichy and Loftus, 1996). First, evaporation rate results in changes of chemical concentration, osmotic pressure, or mechanical stress in the receptor cells (the so-called electrochemical hygrometer model). Second, evaporation causes a temperature differential detected by heat cells and thus the system functions like a psychrometer. Third, changes in cell volume as a result of water uptake or loss are detected, and this constitutes a mechanical model. The latter mechanical hygrometer theory is perhaps the most favoured model, although several aspects of this model remain poorly elucidated (Yokahari, 1978; Tichy and Loftus, 1996), and much of the work is based on only a handful of model organisms (e.g. *Periplaneta*).

Considerable structural variation has been found between insect hygroreceptors in the species that have been investigated (see e.g. Shields, 1994; Tichy and Loftus, 1996; Bland *et al*., 1998; Hunger and Steinbrecht, 1998), and some evidence for functional variation also exists (Tichy and Loftus, 1996; Wolfrum, 1997). Consequently, it has been suggested that several possible models may apply to terrestrial arthropod moisture transduction rather than one ubiquitous system (Tichy and Loftus, 1996; see also Ziegler and Altner, 1995). As Tichy and Loftus (1996) have noted, 'there is still much to be learned' regarding mechanisms of insect hygroreceptor transduction, particularly within an evolutionary and ecological framework.

## 6 Responses to the thermal environment

The thermal environment holds considerable significance for most, if not all levels, of the biological and genealogical hierarchies (Cossins and Bowler, 1987; Gillooly *et al*., 2001, 2005; Allen *et al*., 2002; Hochachka and Somero, 2002; Clarke, 2003, 2006; Evans *et al*., 2005). The form of temperature's effect at various organizational levels, and the behavioural, physiological, and morphological ways in which organisms modify the potential effects of temperature are therefore central to much of physiology and ecology, and continue to engender debate (see e.g. Gillooly *et al*., 2001; Clarke, 2004; Clarke and Fraser, 2004; Gillooly *et al*., 2006; Clarke, 2006). The effects of temperature on an individual insect can be represented in two ways: if resistance responses are under consideration then a thermobiological scale (e.g. Vannier, 1994) is convenient (Fig. 14), while if capacity responses are being considered then a performance curve (Fig. 15) might be more useful, although the distinction between capacity and resistance responses is artificial (Angilletta *et al*., 2002; Chown and Nicolson, 2004). That a mismatch between oxygen supply and demand might be responsible for setting thermal limits in many non-insect species (Pörtner, 2001) nicely makes this point.

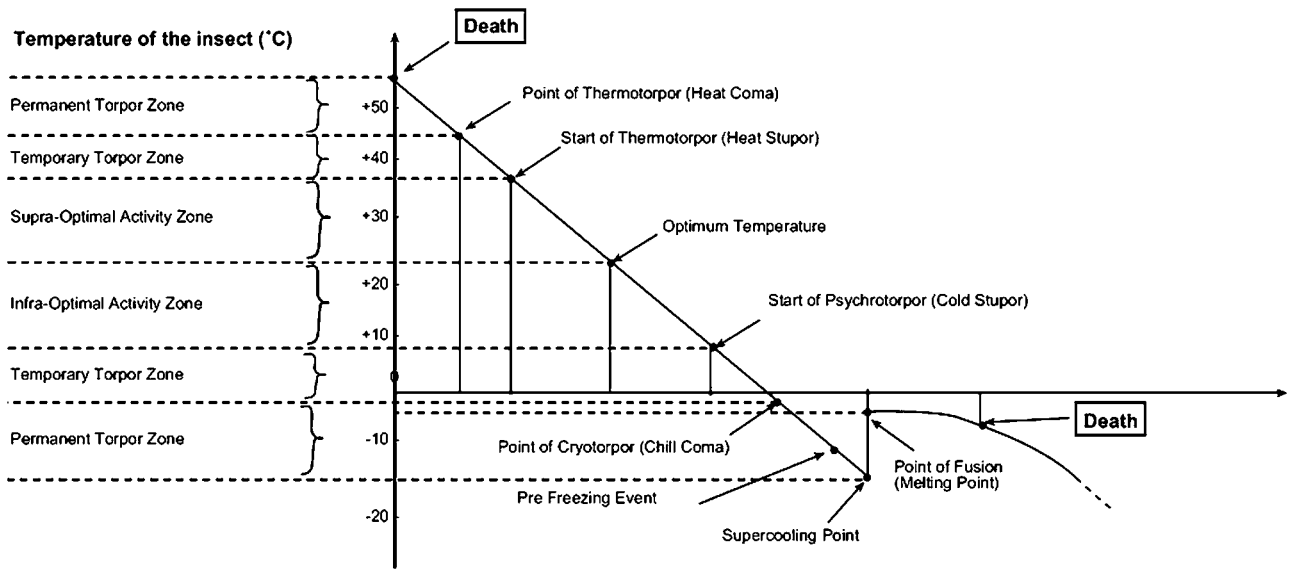



FIG. 14 The thermobiological scale proposed by Vannier (1994). Redrawn with permission from Elsevier.

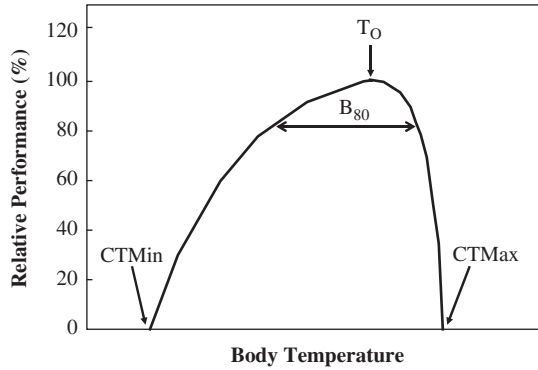



FIG. 15 An idealized thermal performance curve showing the optimum($T_o$), performance breadth($B_{80}$), and critical limits. Redrawn from Angilletta *et al.* (2002, p. 250) with permission from Elsevier.

The literature on the effects of temperature on insects and the responses they mount to counter these effects, or to modify the relationship between the temperatures they experience and their survival probability, is substantial, and has been reviewed recently in several guises (Bale, 2002; Vernon and Vannier, 2002; Sinclair *et al.*, 2003b; Hoffmann *et al.*, 2003b; Chown and Nicolson, 2004; Korsloot *et al.*, 2004). Nonetheless, owing both to the rapid development of molecular tools, and the pressing need to comprehend the likely biological impacts of global change, advances in the field are rapid. Hence, in this section we will provide an appropriate, though not comprehensive, background to the injurious effects of high and low temperatures and individual responses to them at a variety of scales, but will focus more on recent advances in the field.

## 6.1 LOW-TEMPERATURE INJURY

### 6.1.1 *Freezing injury*

One of the most significant physical thresholds for organisms is the transition of water between the liquid and solid phases. Most insects cannot survive freezing, although they typically freeze a few degrees below 0 °C owing to the colligative effects of their body fluids (Zachariassen, 1985). When the temperature of an organism declines below the melting point of its bodily fluids there is a risk of ice formation. Crystallization may take place either by aggregation of water molecules into an ice nucleus (homogeneous nucleation) or via their aggregation around some substance or irregularity (heterogeneous nucleation). When freezing takes place, additional water is added to the nucleus or nuclei, and effectively the animal begins to desiccate. The removal of water from the solution causes an

increase in solute concentration. Progressive concentration of the body fluids may lead to changes in pH, protein denaturation, and alterations of membrane properties, thus affecting electrochemical gradients and transport properties. In addition, cellular shrinkage may occur owing to removal of water from the cells and this may damage the cell membrane to such an extent that it cannot recover following thawing (the critical minimum cell volume hypothesis) (Zachariassen, 1985; Denlinger and Lee, 1998; Ramløv, 2000; Kristiansen and Zachariassen, 2001). The critical minimum cell volume that can be endured likely also sets the lower lethal temperature in insects that are tolerant of freezing (Storey and Storey, 1996). Insects that are able to limit or avoid these injuries, and therefore survive freezing, are also challenged by anoxia because diffusion through ice takes place slowly. Typically, tracheoles are fluid filled when oxygen demand is low, as it is at low temperatures preceding freezing (Irwin and Lee, 2002; Sinclair *et al*., 2004), and the movements that are typical of air sacs and tracheae (Herford, 1938; Westneat *et al*., 2003; Chown and Nicolson, 2004) will also be limited. In consequence, the frozen state is an ischemic one (Morin *et al*., 2005).

Recent work has taken further early investigations of those tissues most sensitive to freezing injury (e.g. Lee *et al*., 1993). Differential sensitivity of tissues to freezing appears to be species specific, although the numbers of species investigated is low. In the Antarctic midge, *Belgica antarctica*, the fat body has the lowest cell viability following freezing, followed by the gut, Malpighian tubules, and salivary glands (Lee *et al*., 2006). Similarly, in *Eurosta solidaginis*, the Malpighian tubules and fat body are more sensitive to freezing than the gut, although in this case the integumentary muscles, haemocytes, and tracheae are most sensitive to low temperatures (Yi and Lee, 2003). Earlier ultrastructural work suggested that the nervous system is especially sensitive to freezing (Collins *et al*., 1997). In the alpine cockroach, *Celattoblatta quinquemaculata*, the gut is most sensitive to freezing, while the Malpighian tubules and fat body are most susceptible to temperatures below the freezing point (Worland *et al*., 2004). In *Chilo suppressalis*, the gut is most sensitive in overwintering larvae, whereas in non-diapausing individuals it is the fat body (Izumi *et al*., 2005).

### 6.1.2 *Chilling injury*

Cold shock, or direct chilling injury is a form of injury that results from rapid cooling in the absence of extracellular ice formation. Direct chilling injury is usually distinguished from the consequences of a long-term exposure to low temperatures, which is known as indirect chilling injury. In both cases, the absence of ice formation distinguishes these kinds of injury from those associated with freezing of an insect's body fluids. Several investigations of insect responses to short- and long-term low-temperature

exposure suggest that these two forms of injury might be different (Chen and Walker, 1994; McDonald *et al*., 1997, 2000). However, comparative assessments of the injuries induced by direct and indirect chilling injury are rare, and much speculation surrounds their relationship and the likelihood that responses to one may alleviate the effects of the other (Sinclair and Roberts, 2005).

Chill coma coincides with the temperature at which the excitability of nerves and muscles is lost (Goller and Esch, 1990; Xu and Robertson, 1994), associated with declining resting potentials. The inability of $Na^+/K^+$-ATPases to function at low temperatures is thought to be a major cause of chill coma (Hosler *et al*., 2000). Although chill coma is reversible, it appears that chilling injury represents ongoing damage to membranes and a marked impact on neuromuscular transmission (Yocum *et al*., 1994; Kelty *et al*., 1996), with downstream effects on reproduction (Denlinger and Lee, 1998; Rinehart *et al*., 2000a; Shreve *et al*., 2004). Direct chilling injury induces fluid-to-gel phase transitions in membranes, which result in separation of membrane proteins and lipids, change membrane permeability, and cause a decline in the activity of membrane-bound enzymes. Direct chilling injury is also a consequence of protein structural changes and denaturation, a decrease in enzyme activity, (Ramløv, 2000; Yocum, 2001), and a possible increase in oxidative stress (Rojas and Leopold, 1996). Fat body and Malpighian tubule cells are particularly prone to direct chilling injury (Worland *et al*., 2004).

Although the mechanisms underlying indirect chilling injury are less well understood than those associated with direct chilling, one recent study has suggested that equilibration of transmembrane ion gradients are important (Koštál *et al*., 2004). In the species investigated, the bug *Pyrrochoris apterus*, absence of energy is not the cause of $Na^+/K^+$-ATPase failure. Rather, the ability of the enzyme to exploit available energy is impaired, suggesting that damage to membrane function at least partially explains indirect chilling injury. Clearly, additional work is required to unveil the mechanisms of indirect chilling injury and their relationship to those responsible for direct injury. However, impacts on membrane pumps, especially the $Na^+/K^+$-ATPase, appear to be common to both. Both direct and indirect chilling injury are likely also a consequence of depletion of substrates (Hoffmann *et al*., 2003b; Renault *et al*., 2004), which may be one reason why a biphasic response to low temperatures is found (Karan and David, 2000). Whatever the cause of the injury, it translates to downstream effects on fitness (e.g. Chakir *et al*., 2005).

Repeated, short-term sublethal low-temperature exposures are a substantial source of injury. In plant-feeding caterpillars of *Pringleophaga marioni*, repeated cooling results in a substantial decline in growth rate, which is a consequence of damage to the gut (Sinclair and Chown, 2005b). How this damage is incurred is not clear, but changes to metabolic rates

during cooling and longer-term alterations in lipid content suggest one way in which this could happen. At the critical thermal minimum, or onset of chill coma (−2 °C), metabolic rate plummets ($Q_{10}$ of $2 \times 10^3$) (Sinclair *et al.*, 2004), which Makarieva *et al.* (2006) interpret as the point at which metabolic control is abandoned (see Section 6.2.2). In caterpillars consuming plant material, oxidation of phenolics can generate reactive oxygen species, which in turn cause membrane lipid peroxidation (Krishnan and Kodrík, 2006). Polyunsaturated fatty acids are especially susceptible to free radical damage (Storey, 1996), and are known to increase in abundance in membranes in response to low temperature (Logue *et al.*, 2000; Hulbert, 2003; Overgaard *et al.*, 2005). If abandoned metabolic control results in cessation of production of anti-oxidants (see Krishnan and Kodrík, 2006), then gut damage may be a consequence of oxidative damage. If this is the likely route of damage to the gut, then it might be expected that in plant chewing, but perhaps not other species, the gut would be most sensitive to low temperature. Currently, insufficient information exists to test this idea.

Failure in organismal performance at low temperatures may also arise at higher levels of organization. The most comprehensive formulation of this idea is the oxygen limitation hypothesis (Pörtner *et al.*, 1998, 2000; Pörtner, 2001, 2002a). In essence, it is thought that in complex organisms (with distinct oxygen acquisition and circulation systems), critical temperatures that affect fitness are not set by cellular level damage, but rather by a transition to anaerobic metabolism. At low temperature these deleterious temperatures (called pejus by Pörtner, 2001) result from insufficient aerobic capacity of mitochondria, and a concomitant decline in ventilation and circulation, which leads to a mismatch between oxygen supply and demand, a drop in aerobic scope, transition to anaerobiosis, and cessation of higher physiological function. At high temperature, insufficient oxygen uptake and distribution by ventilation and circulation to meet mitochondrial demands results in a similar mismatch between supply and demand, and eventual physiological collapse. Pörtner (2001, 2002a) argued that thermal limits in the majority of animals are set by oxygen limitation.

Whether thermal tolerances are set by oxygen limitation has not been widely explored in insects, although several recent studies suggest that cellular level processes are more important. Although temperature–$PO_2$ interactions are found in the eggs of *Manduca sexta*, diffusive supply of $O_2$, rather than ventilation and circulation, is limiting at high temperatures (Woods and Hill, 2004). Declining egg metabolic rates at high temperature are not set by low or falling $O_2$, but either by direct effects of temperature on protein stability or some other, unknown factor. Because the eggs of *M. sexta* are representative of those of many insect species (Woods and Hill, 2004), these findings suggest that oxygen limitation of thermal tolerance is unimportant for insect eggs. In adults of the tenebrionid beetle, *Gonocephalum simplex*, oxygen limitation of upper thermal tolerance does

not appear to be significant either (Klok *et al*., 2004), because changes in ambient $PO_2$ have no effect on thermal tolerance. If oxygen limitation of thermal tolerance is important, thermal tolerance limits should increase at high $PO_2$ and decline at low $PO_2$ (Pörtner, 2001, 2002b).

At low temperatures, it appears that dysfunctional ion pumps are not a consequence of unavailability of ATP, or ventilatory/circulatory problems, but rather as a consequence of the inability of the pumps to utilize ATP (Koštál *et al*., 2004). However, the available data are somewhat equivocal because in the freezing tolerant caterpillars of *Pringleophaga marioni*, individuals that are exposed to a temperature lower than their lethal limit and then thawed have metabolic rates identical to those that have not been killed by freezing, but higher water-loss rates, suggesting that what is lost is central control of processes, rather than cellular-level capabilities (Sinclair *et al*., 2004). In the context of the oxygen limitation of thermal tolerance hypothesis, the data suggest that upper limits are probably set by cellular level damage and insect responses to this damage, while lower limits are set by some combination of cellular and whole-organismal level responses. Decoupling of upper and lower lethal temperatures at a wide variety of levels (Chown, 2001; Chown and Nicolson, 2004) support this conclusion. Nonetheless, these data mostly come from insects at rest. What the situation is in active insects is far from clear, although pronounced effects of hypoxia and hyperoxia on functioning and growth are known from a variety of species (Joos *et al*., 1997; Harrison and Lighton, 1998; Frazier *et al*., 2001).

## 6.2 RESPONSES TO LOW TEMPERATURE

### 6.2.1 *Responses to short-term chilling*

Debate has recently arisen concerning the terminology for the length of exposure of animals to cold (and heat), and the responses they show in consequence (Bowler, 2005; Loeschcke and Sørenson, 2005; Sinclair and Roberts, 2005, see also Spicer and Gaston, 1999). In part, this debate has arisen because both treatments and responses tend to be labelled in the same fashion. Short-term exposures (of minutes to hours) to sublethal conditions are typically termed hardening (Hoffmann *et al*., 2003b) as are the responses shown to these conditions (Bowler, 2005), while cold shock is the stress imposed by these conditions (Denlinger *et al*., 1991). Long-term exposures (days to weeks) to temperatures within the normal viable range of the organism, and the responses shown by the animals, are normally termed acclimation. In the field, animals also respond to low temperatures with substantial long-term alterations to their physiology (Zachariassen, 1985; Storey, 1990; Bale, 2002), which has been termed a 'programmed response to cold' (Chown and Nicolson, 2004) to distinguish it from

shorter-term laboratory treatments. Exposure of immature stages to a given temperature regime may also alter the physiology of later stages, which has been termed developmental plasticity (Piersma and Drent, 2003). All of these changes constitute phenotypic plasticity (Section 4), and the debate has centred largely on whether mechanistic responses (and presumably sources of injury) are similar across the range of responses. The essence of the question is the nature of the time by intensity effect of low-temperature stress. That is, whether the injuries caused by the stress, and the subsequent responses, can be readily divided because of the existence of threshold effects, or whether the time by intensity response space is continuous. Present data do not allow this question to be fully addressed, especially because the full suite of responses has rarely been examined for a given population. However, progress is being made in this area.

In *Drosophila melanogaster*, a comparison has been made of the effects of rearing temperature (developmental effects), a two-day exposure (acclimation), and hardening (a few hours) on fly mortality, chill coma recovery, and recovery during exposure to stress (Rako and Hoffmann, 2006). The responses shown by the flies are complex. Hardening improves survival following a cold shock, but has no effect on chill coma recovery times. Flies reared at 19 °C have lower mortality levels than those reared at 25 °C, and acclimation at 12 °C further reduces mortality in the 19 °C group, but has little effect on the 25 °C group. Flies reared at 19 °C also have longer chill coma recovery times than those reared at 25 °C, and acclimation has a larger effect on the latter than on the former group of flies. When subject to 30 generations of selection, every alternative generation, for decreased chill coma recovery time, this measure of resistance declines, as does mortality following cold shock. However, hardening capability is little effected (though not in males) (Anderson *et al.*, 2005). These studies suggest that the mechanisms underlying longer-term responses of chill coma recovery and survival of low temperature are similar, in keeping with conclusions of earlier work (review in Chown and Nicolson, 2004). However, the mechanisms underlying shorter-term responses probably differ, given that hardening affects mortality but not chill coma recovery, and that the protein synthesis inhibitor, cycloheximide, affects cold shock tolerance, but not tolerance if it is preceded by hardening (Misener *et al.*, 2001, see also Hoffmann *et al.*, 2003b).

Differences in the intensity of stress also affect chill coma recovery. In *Drosophila subobscura*, chill coma recovery time increases with declining temperature in a non-linear fashion. Initially it increases with declining temperature, then remains unchanged, and subsequently increases again (David *et al.*, 2003). This pattern is also evident in *D. melanogaster* (Macdonald *et al.*, 2004; Rako and Hoffmann, 2006). Such a biphasic response suggests that two different mechanisms are responsible for responses to low temperature stress. These effects could be realized in

different ways to result in the response plateau. Two exponential processes could be involved, with a relatively rapid transition from one to the other, or alternatively, one of the processes could be exponential and the other could show a declining sigmoid shape (David *et al*., 2003). The latter is possible only if some process is increasingly damaged at lower temperature up to some maximum level.

What mechanisms underlie acclimation responses to low temperature and the rapid cold hardening response have yet to be fully resolved. Those underlying the longer-term seasonal responses associated with cold hardiness are well understood and the time course and biochemistry of these have been reviewed many times, providing a convenient entry to this large literature (Zachariassen, 1985; Block, 1990; Storey, 1990, 1997; Storey and Storey, 1996, 2004; Denlinger and Lee, 1998; Sømme, 1999; Duman, 2001; Bale, 2002; Chown and Nicolson, 2004). The molecular underpinnings of such mechanisms are now being explored more fully (Morin *et al*., 2005), and the subtleties of responses, including interactions with diapause and their hormonal regulation are being uncovered (Chen *et al*., 2005a; Hayward *et al*., 2005; Tachibana *et al*., 2005). At a biochemical level, mechanisms include the production of low molecular weight cryoprotectants such as polyhydric alcohols (e.g. glycerol, sorbitol), sugars (trehalose), and amino acids such as proline, the production of antifreeze proteins, and either the removal and masking of ice nucelators (in freeze-intolerant species) or the production of protein or lipoprotein ice nucleators in freezing-tolerant species (Chown and Nicolson, 2004).

The mechanisms underlying rapid cold hardening are now beginning to be explored. Initially, it was thought that glycerol plays some role in the response. At least in pharate adults of *S. crassipalpis*, rapid cold hardening is associated with a threefold increase in glycerol levels to 81.4 mM. Although this change is insufficient to have a colligative effect on cold hardiness, glycerol is thought to play a role in protecting membranes against low-temperature damage associated with phase transitions, and in stabilizing proteins (Lee *et al*., 1987a; Kostal *et al*., 2001), although this role has yet to be confirmed. In *S. bullata*, glycerol is produced in response to cold shock and to short-term desiccation and anoxia, but only following a return to higher temperature. In all cases, the glycerol production is not as extensive as in seasonal responses, but it does improve survival (Yoder *et al*., 2006). Moreover, exogenous treatment with glycerol also confers cold hardiness, and ligation of larvae indicates that glycerol production is under central control. These results, and those of Yi and Lee (2004), support the idea that the initial response to cold shock is under local cellular control, and is subsequently complemented by input from the CNS (Yoder *et al*., 2006). They also provide further evidence that short-term responses to low temperature are biphasic. Thus, one, possibly local cellular response generates almost immediate protection, while the second remains active for a longer period.

In other species, such as *D. melanogaster* and the moth, *Lymantria dispar,* glycerol is not produced in response to cold shock (Yocum *et al*., 1991; Kelty and Lee, 1999). In *L. dispar* and in *Sarcophaga crassipalpis*, and the beetle *Leptinotarsa decemlineata*, cold shock results in upregulation of heat-shock protein synthesis (Denlinger and Lee, 1998; Yocum *et al*., 1998; Yocum, 2001). Nonetheless, this typically only takes place once the animals have been returned to a higher temperature (Joplin *et al*., 1990; Rinehart *et al*., 2000b; Yocum, 2001). Moreover, in *D. melanogaster*, Hsps are not synthesized in response to brief low-temperature treatments, but rather only following more extended exposures (Kelty and Lee, 2001; Sejerkilde *et al*., 2003; Overgaard *et al*., 2005; Nielsen *et al*., 2005). In this species, rapid cold hardening is accompanied by changes in the composition of membrane phospholipids fatty acids and an increase in the extent of membrane unsaturation (Overgaard *et al*., 2005). Taken together, these results point to the fact that prevention of damage to membranes, and possibly proteins and the cytoskeleton (see Michaud and Denlinger, 2005) is likely a major component of the rapid cold hardening response, though different routes to such protection are likely, and the intricacies of such mechanisms are far from resolved.

Unsaturation of membranes is a well-documented response to low temperature. It prevents membrane fluid-to-gel transitions (Logue *et al*., 2000; Hochachka and Somero, 2002, Hulbert, 2003), and the role of polyols in stabilizing membranes and proteins is also well established. Recent work has demonstrated that small heat shock proteins protect membranes by improving fluidity of high-temperature melting lipids (Tsvetkova *et al*., 2002), and they may have this role in several species following rapid cold hardening. For example, in *Sarcophaga crassipalpis*, RNA interference of hsp23 causes a significant and substantial reduction of survival of cold shock following hardening. By contrast RNAi of hsp70 has little effect on survival following hardening, leaving the upregulation of Hsp70 during rapid cold hardening in the species unexplained (Michaud and Denlinger, 2005; Chown and Storey, 2006). Whatever the mechanisms underlying rapid cold hardening finally turn out to be, it is clear that the protective effects of the response to rapid cold hardening extend not only to survival, but also to several other components of fitness (Rinehart *et al*., 2000a).

The mechanisms that underlie improvement of survival following longer-term exposures to low temperature, such as those used during investigations of acclimation (several days) have not been investigated to the same extent. Past overviews have tended not to draw a distinction between investigations of the rapid hardening and acclimation responses (e.g. Chown and Nicolson, 2004). Low-temperature treatments of several days result in expression of Hsps in *Drosophila melanogaster*, *Lymantria dispar,* and in several species of *Drosophila* (Burton *et al*., 1988; Denlinger *et al*., 1992; Goto and Kimura, 1998; Goto *et al*., 1998). This expression typically

takes place only following a return to high temperatures. Recent work on *Drosophila* has suggested that increases in Hsp70 following long-term cold acclimation may have to do with repair of damage induced both by low temperature and by re-heating (Goto and Kimura, 1998; Sejerkilde *et al*., 2003; Nielsen *et al*., 2005; Overgaard *et al*., 2005). Several studies have demonstrated that alternating temperatures (i.e. cessation of chilling and return to higher temperatures) improve the survival of chilling (Chen and Denlinger, 1992; Coulson and Bale, 1996; Hanc and Nedved, 1999; Renault *et al*., 2004), and this may be a consequence of the synthesis of Hsps and possibly also polyhydric alcohols at the higher temperatures. Additional responses to longer-term cold exposure include elevation of energy reserves (Chen and Walker, 1994; Misener *et al*., 2001). However, the role of polyhydric alcohols has not been well explored.

### 6.2.2 *Programmed responses to cold*

Seasonal changes in physiology in anticipation of declining environmental temperatures, and the variety of strategies which insects employ to overcome low temperatures in temperate and polar regions, have received much attention (Bale, 1987, 1993, 2002; Sinclair, 1999; Vernon and Vannier, 2002; Sinclair *et al*., 2003b; review in Chown and Nicolson, 2004). What is much less clear is what circumstances might promote each of the various strategies, or what their fitness costs and benefits are (Block, 1991; Voituron *et al*., 2002). It is widely accepted that the basal response shown by insects to freezing is freeze intolerance (Vernon and Vannier, 2002). Therefore, freeze tolerance is a derived strategy, though it probably originated several times (Sinclair *et al*., 2003a). Early work (see Zachariassen, 1985) pointed to the importance of freezing tolerance in areas with extremely low temperatures, especially given that supercooling in freeze-intolerant species is a metastable state. This work also suggested that freezing tolerance promotes cold hardiness in insects that retain ice-nucleating agents in their haemolymph and gut, such as those exposed to regular freezing events. These early ideas were further developed to show that regular freeze-thaw events associated with environmental unpredictability are likely the major environmental factor selecting for moderate freeze tolerance (Sinclair *et al*., 2003a; Sinclair and Chown, 2005a). Thus, the proposed advantages to freezing tolerance over freeze intolerance can be summarized as follows:

- Nucleation Hypothesis I: Non-zero risk of freezing during long-term exposure in freeze-intolerant individuals.
- Nucleation Hypothesis II: Short-term risk of inoculative freezing in freeze-intolerant individuals, especially in moist environments.
- Desiccation avoidance hypothesis: Supercooled insects are in vapour pressure deficit if surrounded by ice.

- Extreme survival hypothesis: At very low temperatures the supercooled state may be stable for short periods only.
- Energy conservation hypothesis: Freezing reduces metabolic rate and the latter is apparently insensitive to changes in temperature in frozen animals.
- Environmental variability hypothesis: Freezing tolerance enables animals to survive cold snaps at any time without metabolically costly synthesis of additional cryoprotectants, and enables them to take advantage of warm spells to continue with growth and development.

To some extent these hypotheses do not recognize the complexity of responses, which may include mixed strategies and changes in strategies following exposures to low temperature (Kukal and Duman, 1989; Bale *et al.*, 2001; Brown *et al.*, 2004). Moreover, the remaining qualitative hypotheses which, while useful, lack the rigour of the models applied to many other problems in insect life-history theory (see Roff, 2002).

One attempt to place the costs and consequences of cold hardiness strategies on a more quantitative footing is the energetic model of cold hardiness developed by Voituron *et al.* (2002). They assumed that the strategy adopted is the one that maximizes fitness as measured by available energy at the end of winter, which can be represented by the fitness differences of the two strategies, $\Psi$, from the equation:

$$\Psi = (W_0 - S_T)\left[1 - \left(\frac{N}{N_{\max}}\right)^{\theta}\right] - W_0 + S_A + aTN \qquad (1)$$

where $W_0$ is the metabolizable energy reserve at the start of winter, $S_T$ the energetic cost of a freezing tolerance strategy, $N$ the number of freezing days, $N_{\max}$ the maximum number of freezing days before death, $S_A$ the energetic cost of freeze intolerance, $a$ the sensitivity of freeze intolerance to climate (energy required to produce reliable cyroprotection for $NT$), $T$ the cold intensity, and $\theta$ the shape of the change in fitness (or $W_T$ – energy available at end of winter) of a freezing tolerant individual as $N_{\max}$ is approached.

When $\Psi > 0$, then freezing tolerance will be favoured and when $\Psi < 0$, freeze intolerance is favoured. Analytical and simulation work by Voituron *et al.* (2002) has demonstrated that freezing tolerance is favoured by low stress associated with freezing, low initial energy content, high number of freezing days, and a high value of $\theta$. By contrast, freeze intolerance is favoured by a low number of freezing days, low stress associated with supercooling, low sensitivity to climate, and high initial energy content. The model also indicates that harsher conditions should favour a mixed strategy.

The majority of these outcomes appear to be in keeping with empirical findings, especially for extreme strategies such as strong freezing tolerance and freeze avoidance (Chown and Nicolson, 2004). As a result, it is a much needed and useful first step towards a quantitative understanding of the costs and benefits of each of the strategies. Nonetheless, as recognized by Voituron *et al.* (2002), the model is less able to deal with other strategies and requires further development to do so (see also Sinclair *et al.*, 2003a). Additionally, several of the basic assumptions made by the model either have been poorly explored, or remain theoretical constructs only, in need of empirical evaluation.

The value of $\theta$, or the form of the relationship between number of days remaining until $N_{\max}$ and $W_T$, has a significant influence on the likelihood that freezing tolerance will be favoured, but has not been explored at all. If frozen individuals are largely anoxic (Storey and Storey, 1996; Morin *et al.*, 2005), then duration of survival is likely to be a function not of energy stores, but rather of the extent of damage owing to chaotic biochemical reactions (Knickerbocker and Lutz, 2001; Milton *et al.*, 2003a; Makarieva *et al.*, 2006). Anoxic organisms die after the cumulative energetic yield of chaotic anoxic biochemical processes has passed c. 70–100 kJ (kg dry mass)$^{-1}$, which means that the more effectively an organism can suppress the accumulation of disorder, the longer it will survive (Fig. 16). Consequently, it might be argued that $\theta$ should typically have a value substantially less than 1, which is a situation unlikely to promote freezing tolerance (Voituron *et al.*, 2002, p. 262). The energy available to an organism in advance of freezing has little influence on whether or not a freezing tolerant strategy will be followed, by contrast with the assumptions of the model. Rather, it is the extent of biochemical conservation of tissues in advance of the anoxic condition, or limitation of damage by chaotic reactions, that is of most significance (Makarieva *et al.*, 2006).

Another assumption of the modelling approach is temperature independence of metabolic costs in frozen animals (Voituron *et al.*, 2002, p. 257). The logic used is that anoxic individuals meet their energetic demands through anaerobic pathways, and the energy cost of frozen animals is therefore temperature independent. Unfortunately, empirical evidence for this idea is at best weak. Although several studies have measured metabolic rates of frozen and supercooled insects, they are confounded by the use of closed system respirometry, which makes detection of activity difficult (reviewed in Sinclair *et al.*, 2004). In addition, estimates of energy expenditure at very low temperatures in frozen insects can probably only be made reliably using calorimetric (see Hansen *et al.*, 2004, 2006) or biochemical methods, owing to the fact that metabolism is either anaerobic and even if it is not, diffusion through ice is extremely low. Nonetheless, evidence gained from other studies of species experiencing anoxia suggests that the relationship between metabolic rate (which in this instance is like a

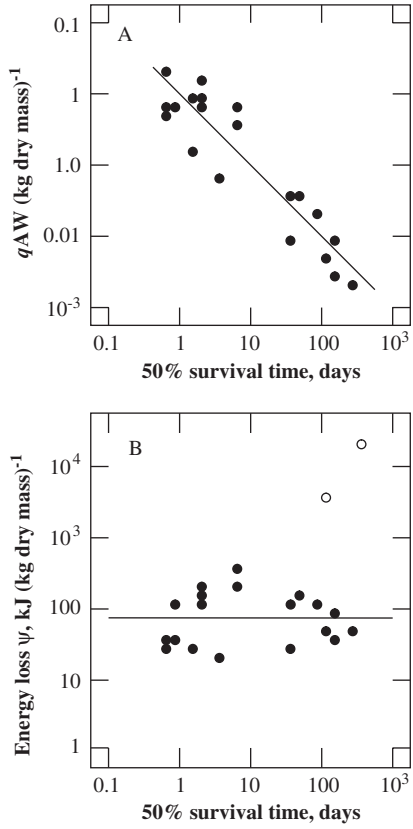



FIG. 16 Metabolic rate, energy loss, and survival time under anoxic conditions. (A) Mass-specific rates, $q$A, of energy dissipation by organisms capable of surviving more than half a day of anoxia. (B) Energy loss ψ during anoxia is independent of survival time (filled circles). The open circles indicate normoxic energy losses of bears during hibernation and ticks during prolonged starvation. Redrawn from Makarieva *et al.* (2006, p. 90).

measure of chaotic biochemical reactions) and temperature is positive, with a $Q_{10}$ of approximately 2.8 (Makarieva *et al.*, 2006). Therefore, it appears that several assumptions made in the model developed by Voituron *et al.* (2002) require further exploration, as does consideration of the reasons for the development of the two major cold hardiness strategies.

One possibility is that the freezing-tolerance and freeze-intolerance responses represent the two major alternatives for surviving stress, i.e. abandoned metabolic control and minimum metabolic control, respectively (Makarieva *et al.*, 2006). In the case of abandoned metabolic control, the organism maximally protects its cellular structures against degradation and then switches off most other metabolic processes. The disadvantage of this

strategy is that no matter how low, the rate of spontaneous degradation of structures is never zero. However, in this case, repair is not possible until favourable conditions return. Therefore, once a critical threshold has passed (which appears to be c. 100 kJ (kg dry mass)$^{-1}$), the animal dies. Survival time is dependent on the rate of spontaneous degradation, and decreases with increasing temperature because degradation has a $Q_{10}$ of approximately 2.8. In addition, the energy losses tolerated in the regime of abandoned metabolic control are much lower than those tolerated under minimum metabolic control. In the case of minimum metabolic control, the organism survives the stress by continually sustaining order at a minimum metabolic level, which is independent of both body mass and temperature. The period of survival depends on energy stored by the organism. Hence, much greater rates of energy loss per unit body mass can be tolerated because the loss is non-random and occurs in storage tissues without threatening organismal integrity. For example, hibernating bears can tolerate losses in the region of 16 000 – 40 000 kJ (kg dry mass)$^{-1}$, and starved ticks can tolerate energy losses of 4300–20 000 kJ (kg dry mass)$^{-1}$. Nonetheless, some damage may accumulate, which might also account for the periodic arousal that is typical of many hibernating organisms (Makarieva *et al*., 2006, see also McNab, 2002).

At least some evidence suggests that this categorization of cold hardiness strategies is appropriate. In freeze-tolerant insect species, metabolic rate drops rapidly just in advance of freezing or as it occurs (Lundheim and Zachariassen, 1993; Irwin and Lee, 2002; Sinclair *et al*., 2004), which Makarieva *et al*. (2006) have interpreted as the point at which metabolic control is abandoned. Furthermore, in the freeze-tolerant *Eurosta solidaginis,* metabolic rate (which is largely anoxic in the frozen state, Joanisse and Storey, 1994a, 1996) is strongly related to temperature in the frozen state, and increases in temperature reduce survival, in keeping with the expectations of abandoned metabolic control (Irwin and Lee, 2000, 2002, 2003; Irwin *et al*., 2001). Moreover, antioxidant enzymes show a decline in winter (Joanisse and Storey, 1996), and this species shows no overwinter heat-shock protein response to cold stress (Lee *et al*., 1995), indicating that most metabolic processes are shut down (although accumulation of some polyols may continue and anaerobic metabolism certainly proceeds – see Joanisse and Storey 1994a,b). Degradation of mitchondria over winter in the strongly freezing-tolerant caterpillars of *Gynaephora groenlandica* (Kukal *et al*., 1989) is also indicative of abandoned metabolic control, especially since these structures might be most susceptible to chaotic metabolic reactions (Makarieva *et al*., 2006).

Whether freeze intolerance represents minimum metabolic control is more difficult to ascertain. Previous studies have claimed a strong relationship between temperature and metabolic rate in the supercooled state, although the technical approach used precludes assessment of the influence

of insect activity (see Sinclair *et al*., 2004). However, some findings support the idea that aerobic metabolism with ongoing damage repair characterizes freeze intolerance. Thus, in the goldenrod gall moth, *Epiblemma scudderiana*, metabolism is clearly aerobic (Joanisse and Storey, 1994b, 1996), and in several other freeze-intolerant species, a heat-shock protein response is shown (Denlinger, 2002; Chen *et al*., 2005a), suggesting ongoing damage control and repair. In diapausing, freeze-intolerant pupae of flesh flies, periodic increases in metabolic rate (infradian cycles) are apparent (Denlinger *et al*., 1972), suggesting that minimum metabolic rates are unable to sustain all damage repair and that periodic increase in metabolism are required to do so. Similar oscillating patterns have been found in overwintering individuals of *Megachile rotundata* (Yocum *et al*., 2005).

Thus, despite the fact that a large literature exists on insect responses to winter cold, it is clear that much remains to be done to understand the environmental and life-history contexts of these responses. For example, the effects of moulting on SCPs is now only beginning to be understood in some species, and it appears that much of the variation in the frequency distributions of SCPs, which has long occupied physiologists, is likely non-adaptive (Fig. 17) (Worland, 2005; Worland *et al*., 2006). Nonetheless, an excellent start has been made at understanding the life-history contexts of cold hardiness responses especially in the context of the metabolic costs of the strategies. Indeed, interactions between metabolism and cold hardiness have not been explored to any large extent, despite the fact that they are likely to prove significant for understanding the evolution of low-temperature tolerance (Voituron *et al*., 2002; see also Hoffmann *et al*., 2005b).

## 6.3 RESPONSES TO HIGH TEMPERATURE

### 6.3.1 *High-temperature injury*

High-temperature injury results from disruption of the structure of membranes (Hochachka and Somero, 2002) and in consequence their function, especially those of neurons (Robertson, 2004a; Klose and Robertson, 2004). The ways in which the structure of membranes are disrupted by high temperatures have been reviewed in considerable detail (Hochachka and Somero, 2002), and will not be considered here. High temperature also results in alterations in the cell microenvironment, and especially affects the cytoskeleton (Klose and Robertson, 2004) and pH (Denlinger and Yocum, 1998), perturbation of protein structure, and DNA lesions (Somero, 1995; Feder, 1999). Intense thermal stress can perturb the structure of an organism's proteins. During normal cellular functioning, proteins are generally folded, but may be unfolded during transport, synthesis of polypeptides, and assembly of multimeric proteins. Stress may also

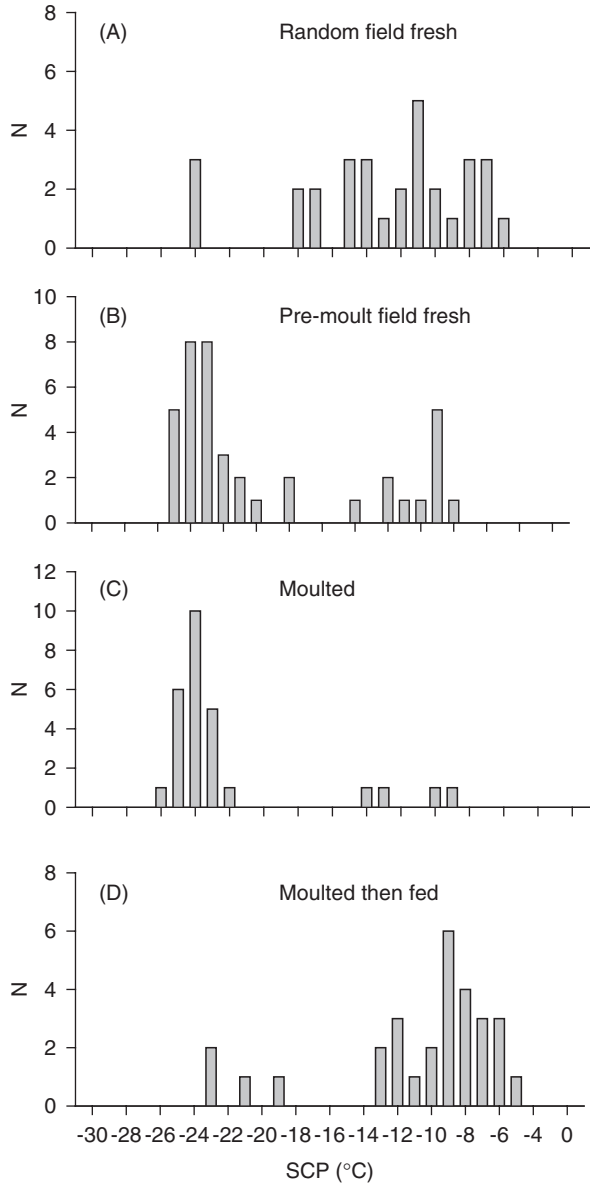



FIG. 17 Supercooling point (SCP) distributions from the springtail *Ceratophysella denticulata* on sub-Antarctic Marion Island from (A) an 'arbitrary' field sample, (B) pre-moulting animals from the same main sample, (C) recently moulted animals, and (D) recently moulted animals that had been fed for one day (10 °C). Note the substantial decline in supercooling point associated with the moulting process and the increase thereof following feeding. Redrawn from Worland *et al.*, 2006.

result in unfolding. In this unfolded state, exposed amino acid side groups, especially hydrophobic residues, can lead to interactions between these 'non-native' proteins and folded proteins, inducing the latter to unfold. The result is irreversible aggregations of unfolded proteins. These unfolded proteins reduce the cellular pool of functional proteins and may also be cytotoxic (Feder, 1996, 1999; Feder and Hofmann, 1999; Kregel, 2002; Korsloot *et al*., 2004).

Neuronal phenomena are characterized by two main types of thermal sensitivity. The first is a consequence of temperature-dependence of conduction, action potential duration, and synapse functioning. In turn these reflect temperature dependence of activation and inactivation of ion channels, which are the result of conformational changes in protein structure. When a neuronal parameter is made ineffective by high or low temperature then limits are set (Robertson, 2004a). The second is a consequence of a high thermal dose (duration and intensity of stress), which causes disturbance or damage eventually leading to failure of neuronal function. A primary site of thermal damage is the cytoskeleton, which contributes to processes underlying synaptic plasticity (Klose *et al*., 2004). Stress causes disruption of actin microfilament integrity, resulting in dissociation from membranes, disassembly of microtubules, and collapse of intermediate filaments towards the nucleus (Klose and Robertson, 2004). Synaptic damage is also a major consequence of thermal stress (Karunanithi *et al*., 2002; Robertson, 2004a), and damage to neuronal functioning takes place as a consequence of a decrease in amplitude and duration of the action potential. The latter is a consequence of rapid activation of $K^+$ currents, which overwhelm the $Na^+$ current before the latter can develop fully (Robertson, 2004a), as well as an extracellular accumulation of $K^+$ (Robertson, 2004b). These effects are of considerable significance given the importance of the nervous system in enabling an organism to sense and respond to its environment (Klose and Robertson, 2004).

The damage wrought by sublethal thermal stress affects development, muscular contraction, flight ability, fertility, and several other processes at higher organizational levels (Denlinger and Yocum, 1998; Rohmer *et al*., 2004; Chakir *et al*., 2005; Krebs and Thompson, 2005; Jørgensen *et al*., 2006). The organizational level at which thermal stress has most effect has not been fully resolved. However, it seems likely that it is not at the level of acquisition and transport of oxygen (i.e. oxygen limitation of thermal tolerance) as has been suggested by Pörtner (2001) (see Section 6.1.2). That insect upper thermal tolerances show relatively little geographic variation, at least by comparison with lower lethal limits, and that thermal limits are invariant with changing oxygen concentrations provide support for this idea (Chown, 2001; Klok *et al*., 2004). However, the number of species investigated in the latter case remains small, and the response to a prolonged stress has not been assessed.

### 6.3.2 *Basal responses*

The temperature at which heat induces injury and/or death varies both through space and in time. Because differences in methods of measurement usually assess different traits (e.g. knockdown resistance vs. survival), resulting in dissimilar outcomes, it is difficult to reach general conclusions regarding upper thermotolerance limits (see Chown and Nicolson, 2004). However, in insects they generally do not exceed about 53 °C, and are usually not much lower than 30 °C, although these values depend on the trait being measured. There are examples of very low tolerance levels in some species such as alpine grylloblattids, and tolerance may increase dramatically in dormant, virtually anhydrobiotic stages such as eggs. Ignoring these extreme values, substantial variation in tolerances remains, although it is typically less than that found for lower lethal limits (Addo-Bediako *et al.*, 2000; Chown, 2001; Hoffmann *et al.*, 2005a).

The physiological basis of this variation in thermotolerance is much less clear. It has been suggested that constitutively expressed heat shock proteins (Hsps, see later) might be responsible for both survival of potentially lethal temperatures and for improved knockdown resistance (McColl *et al.*, 1996; Gilchrist *et al.*, 1997; but see also Nielsen *et al.*, 2005). Alternatively, alterations in cell membrane composition or changes in allozymes or their concentrations might also be involved (Hochachka and Somero, 2002). One explanation for increased basal thermotolerance is the cost of a low-level, induced-stress response. Continuous expression of heat shock proteins reduces survival and fecundity, inhibits growth and thus affects development time (Krebs and Loeschcke, 1994; Feder and Krebs, 1998; Feder, 1999), and impairs locomotion (Robertson, 2004b). It also acts as a substrate sink, and interferes with cellular functioning (Zatsepina *et al.*, 2001). Consequently, at high temperatures there might be a considerable premium for reduction of this response, and probably an increase in basal thermotolerance allowing the organisms to cope with what are otherwise potentially injurious temperatures. This basal thermotolerance may be a consequence of constitutively expressed hsps (Lansing *et al.*, 2000), the presence of osmolytes (Wolfe *et al.*, 1998), or alterations in membranes and allozymes (Zatsepina *et al.*, 2001).

### 6.3.3 *Induced tolerance and its underlying mechanisms*

It has long been appreciated that injury caused by high temperature can be ameliorated by prior exposure to a sublethal, or moderately high temperature (Denlinger *et al.*, 1991; Hoffmann and Watson, 1993; Robertson *et al.*, 1996). This acclimation response lasts for several hours, but is nonetheless transient (Krebs and Loeschcke, 1995). Induced thermotolerance responds strongly both to artificial selection (Krebs and Loeschcke,

1996), and to laboratory natural selection (Cavicchi *et al*., 1995), and it is clear that this trait shows considerable genetic variation (Krebs and Loeschcke, 1997; Loeschcke *et al*., 1997).

The expression of heat shock proteins, which act as molecular chaperones to proteins, is now recognized as one of the most widespread and conserved responses to thermal and other stresses (Feder and Hofmann, 1999; Kregel, 2002). Molecular chaperones interact with the unfolded proteins to minimize their harmful effects by binding to the exposed side groups, preventing unfolded proteins from interacting. In an ATP-dependent manner, they also release the proteins so that they can fold properly, and target proteins for degradation or removal from the cell (Parsell and Lindquist, 1993). Hsps have a significant role in protecting cytoskeletal integrity during thermal stress, and are also of considerable significance for retention of neuronal functioning (Karunanithi *et al*., 1999; Klose *et al*., 2004). These heat shock proteins comprise several families that are recognized by their molecular weight, and include Hsp100, Hsp90, Hsp70, Hsp60, and a family of smaller proteins (Denlinger *et al*., 2001). The roles of these families differ, and the smaller Hsps also have a function in membrane stabilization (Tsvetkova *et al*., 2002).

The best known of these families in insects is Hsp70, especially because of its dramatic increase in *Drosophila* in response to high-temperature stress. Conclusive demonstrations of the association between Hsp70 expression and thermotolerance have come from investigations of isofemale lines and genetically engineered strains of *D. melanogaster* (Krebs and Feder, 1997; Feder and Krebs, 1998; Feder and Hofmann, 1999), as well as investigations of *Sarcophaga crassipalpis* (Denlinger and Yocum, 1998), and the locust nervous system (Robertson, 2004a,b). Heat shock is also known to induce expression of Hsp70 and to confer thermotolerance and undertake chaperoning roles in several other insect species such as locusts, whiteflies, beetles, moths, ants, fruit flies, and parasitic wasps (Denlinger *et al*., 1991, 1992; Gehring and Wehner, 1995; Thanaphum and Haymer, 1998; Maisonhaute *et al*., 1999; Salvucci *et al*., 2000; Landais *et al*., 2001; Qin *et al*., 2003; Neargarder *et al*., 2003; Mahroof *et al*., 2005). Therefore, it seems likely that Hsp70 will be identified as a common component of the heat shock response in most taxa, although the nature and complexity of the response is likely to vary. Nonetheless, ongoing expression of Hsps can have significant deleterious effects (see above). This may explain the decline in the expression of Hsp70 and inducible thermotolerance when either laboratory strains (Sørenson *et al*., 1999; Lerman and Feder, 2001) or wild populations (Sørenson *et al*., 2001; Zatsepina *et al*., 2001; Qin *et al*., 2003) evolve at high temperatures.

In aphids and whiteflies, the synthesis of protective osmolytes, specifically polyhydric alcohols, also confers thermotolerance (Wolfe *et al*., 1998; Salvucci, 2000; Salvucci *et al*., 2000). Sorbitol accumulates to levels as high

as 0.44 M within 3 h of exposure to high temperatures in the whitefly, *Bemisia argentifolii*, and appears to serve the same protective role as heat shock proteins. At physiological concentrations, sorbitol increases the thermal stability of proteins by stabilizing their structure and preventing heat-induced aggregation, thus maintaining catalytic activity at high temperatures (Salvucci, 2000). If the insects are deprived of nutrients, sorbitol production declines and heat shock proteins assume greater importance in protecting proteins against thermal stress. Nonetheless, it appears that sorbitol is routinely produced as a rapid response to high temperature, via an unusual synthetic pathway involving fructose and an NADPH-dependent ketose reductase.

Further responses to high temperature stress involve modulation of potassium conductance in the neuronal system. Prior heat shock improves tolerance of stress in a variety of nervous tissue preparations (see Klose and Robertson, 2004; Robertson, 2004a,b for review). At least one consequence of this heat shock is a reduction in whole cell $K^+$ conductance, which is mimicked by the application of serotonin (5-hydroxytryptamine) (Ramirez *et al*., 1999; Wu *et al*., 2002). This results in an increase in action potential duration and a reduction in the extracelleular accumulation of potassium ions. Both have substantial effects on neuronal functioning (Robertson, 2004b). Nonetheless, the response is complex and differs both between tissues and between species (Robertson, 2004a). Prior heat shock reduces recovery time in the ventilatory central pattern generator following thermal stress, and it seems likely that heat shock somehow activates the $Na^+/K^+$-ATPase, although how this happens has not been investigated. Nonetheless, it is clear that prior heat shock confers substantial thermal tolerance across the nervous system, at the presynaptic, synaptic and axonic levels.

## 6.4 RELATIONSHIPS BETWEEN HIGH- AND LOW-TEMPERATURE TOLERANCE

### 6.4.1 *Organismal level responses*

Given that both polyols and heat shock proteins are expressed in response to cold and heat shock, and that pretreatment at a high temperature increases tolerance of cold shock (Chen *et al*., 1991; Sinclair and Chown, 2003), and *vice versa* (Sejerkilde *et al*., 2003), depending on the species, it might seem that the responses to heat and cold are similar. However, although heat shock proteins are synthesized rapidly in response to both cold and heat treatments, considerable differences exist between these two responses. First, the time course of the response differs. Hsps are often not produced in response to rapid cold hardening, but are expressed following cold acclimation, and usually they are produced only once individuals return to higher temperatures. By contrast, during heat shock, Hsps are synthesized during the stress. Second, the duration of the response differs

dramatically between the two forms of shock. Usually, synthesis of Hsps in response to high temperature is brief and ceases almost immediately on cessation of the stress (Yocum and Denlinger, 1992), while in response to low temperature, Hsp synthesis may continue for days (Yocum *et al.*, 1991). Third, during heat shock, normal protein synthesis is almost entirely replaced by stress protein synthesis, whereas following a cold shock normal protein synthesis and the production of stress proteins occur concurrently. Finally, upregulation of serine proteinase genes associated with immune function also differs between cold and heat shock (Chen *et al.*, 2005b).

Nonetheless, other responses, such as stabilization of membranes by polyols and modulation of membrane pumps may be common to both high- and low-temperature responses, as is the improvement to stress resistance under alternating temperature regimes (Pétavy *et al.*, 2001). In addition, central regulation of responses involves similar mechanisms. Thus, while the initial response to cold and heat shock takes place at the cellular level (Hochachka and Somero, 2002; Yi and Lee, 2004), subsequent responses are centrally regulated (Denlinger and Yocum, 1998; Yoder *et al.*, 2006). Hormonal responses may also turn off downstream functions, such as reproduction, that would otherwise be negatively affected by thermal stress (Pszczolkowski and Chiang, 2000; Gruntenko *et al.*, 2000, 2003a,b; Irwin *et al.*, 2001; Pszczolkowski and Gelman, 2004).

Interactions between hormonal regulation of insect development and stress resistance have been especially well explored for cold hardiness and diapause. In the flesh fly, *Sarcophaga crassipalpis*, non-diapausing pupae are much more sensitive to low temperature than pupae in diapause, which can survive prolonged exposure to temperatures approaching their SCP (c. −23 °C) (Lee and Denlinger, 1985; Chen *et al.*, 1987; Lee *et al.*, 1987b). Ecdysteroid titre drops rapidly at the onset of diapause and it seems likely that genes associated with the action of these hormones are essential for regulating diapause (Denlinger, 2002; Hayward *et al.*, 2005). Transcripts for heat shock protein 90 (hsp90) are downregulated during diapause, and their expression is likely controlled by 20-hydroxyecdysone (Rinehart and Denlinger, 2000). Exposure to both cold and heat shock results in upregulation of hsp90, and exposure to cold, but not heat results in upregulation of heat shock cognate 70 (hsc70) (Rinehart *et al.*, 2000b). By contrast, hsp23 and hsp70 are upregulated at the start of diapause and downregulated rapidly when diapause is terminated (Yocum *et al.*, 1998; Denlinger *et al.*, 2001). During diapause neither heat shock nor cold shock result in further upregulation of these heat shock proteins, possibly as a consequence of upregulation of hsp90.

Given that the continued expression of heat shock proteins is known to be deleterious, their continued upregulation during diapause in *S. crassipalpis* initially appears remarkable. However, cell cycle arrest plays an important role in diapause in *S. crassipalpis*. Therefore, if the majority of negative

effects of Hsp expression have to do with reduced cellular growth and differentiation, Hsps may have little adverse effect during diapause and may even assist in the maintenance of diapause (Hayward *et al.*, 2005), as well as serving to protect diapausing individuals from thermal and other stresses. The downregulation of hsp90 at the onset of diapause, and its upregulation following diapause termination, or in response to heat or cold shock, is also readily comprehensible within this framework. Hsp90 keeps unstable proteins ready for activation until they are stabilized during signal transduction. Thus, given relative cell inactivity during diapause, Hsp90 is unlikely to be required, but because of its ability to stabilize proteins, it remains responsive to thermal stress. However, this pattern of expression is not common to all insect species (e.g. Goto *et al.*, 1998; Goto and Kimura, 2004; Chen *et al.*, 2005a; Tachibana *et al.*, 2005). Therefore, the role of Hsps during diapause, and their hormonal regulation deserve further exploration.

### 6.4.2 *Geographic variation*

At higher levels of organization, substantial differences between tolerance to high and low temperatures are particularly evident. Geographic variation in response to cold and heat shock have been investigated in several species (e.g. Goto and Kimura, 1998), but the comparison of flesh flies from tropical and temperate areas made by Chen *et al.* (1990) is one of the most comprehensive. While all the species show an inducible tolerance to heat shock, only the species from temperate and alpine areas show rapid cold hardening. As might be expected, basal tolerance of cold is greater in the temperate and alpine species than in the tropical ones, but this is true also of basal heat tolerance. Although this appears somewhat unusual, it should be kept in mind that mid-latitude areas are often characterized by very high temperatures (Sømme, 1995), and that global variation of absolute maximum temperatures is much less than that of absolute minima.

This difference in global temperature variation lies at the heart of similar large-scale patterns in insect thermal tolerances. Latitudinal variation in upper lethal limits, though significant (a range of about 30 °C), is much less pronounced than spatial variation in lower lethal temperatures (a range of about 60 °C) (Addo-Bediako *et al.*, 2000). Similar patterns are found across smaller geographic ranges both within and between species (e.g. Chown, 2001; Ayrinhac *et al.*, 2004; Hoffmann *et al.*, 2005a; Terblanche *et al.*, 2006), and in a variety of stages (e.g. Shintani and Ishikawa, 1999; Jing and Kang, 2003; Wang and Kang, 2005). Similar clines in genes associated with the response to thermal stress are now also being demonstrated (e.g. Bettencourt *et al.*, 2002; Frydenberg *et al.*, 2003). Much of the variation within species is environmentally induced. In other words, common garden experiments reveal that differences among populations can largely be accounted for by phenotypic plasticity. The significance of phenotypic

plasticity in shaping responses to the environment has also been demonstrated for altitudinal clines (e.g. Klok and Chown, 2003), where many of the intraspecific thermal tolerance patterns, and indeed interspecific patterns, are similar to those found across latitude (e.g. Collinge *et al*., 2006), although exceptions can be found (e.g. Sørensen *et al*., 2005). Laboratory selection experiments have revealed similar, differential responses to heat and cold (reviews in Chown, 2001; Chown and Storey, 2006).

## 6.5 LOW TEMPERATURE, DEHYDRATION, AND STARVATION

It has been widely accepted for at least the past decade that a physiological link exists between an insect's ability to withstand cold and its ability to survive dehydration (reviewed in Ring and Danks, 1994; Block, 1996; Denlinger and Lee, 1998; Danks, 2000; Chown and Nicolson, 2004). This is at least partly a consequence of the recognition that the damage caused by desiccation and by freezing is similar (for review see Storey and Storey, 1996). Indeed, cellular hydration state probably acts as a trigger for many of the mechanisms that enable survival of subzero temperatures and dry conditions (reviewed in Schliess and Haüssinger, 2002). It is widely accepted that cell and whole-animal regulatory processes that are affected by hydration state, including processes directly resulting in cell death, are directly influenced by cell volume changes (Chamberlin and Strange, 1989; Parker, 1993; Schliess and Haüssinger, 2002), although this evidence has come primarily from mammalian tissues (though see Chamberlin and Strange, 1989). Changes in cell hydration state may be sensed by a variety of mechanisms including stretch-activated ion channels, cytoskeletal elements, and changes in membrane structure (reviewed in Chamberlin and Strange, 1989; Parker, 1993). The interactions among cell volume, osmotic status and stress responses are complex. Changes in cell volume associated with dehydration and rehydration, and which are mediated to some degree by the osmotic state of the cell (Parker, 1993; Lang *et al*., 1998; Schliess and Haüssinger, 2002), can also induce other stress responses (Schliess *et al*., 1999). Cell hydration state is also closely coupled with oxidative stress responses. In the case of the latter, there is some evidence to suggest that oxidative stress can be converted into osmotic stress and that the converse may also be true (Qin *et al*., 1997; Schliess and Haüssinger, 2002), although this type of response may be restricted to specific mammalian tissues or selected cell types. The critical minimum cell volume is widely acknowledged to be a threshold for cellular functioning (Storey and Storey, 1996), and iso-osmotic declines in cell volume can directly result in apoptotic cell death (Schliess and Haüssinger, 2002). Typically, the association of these stress responses with cellular hydration status, either by volume or cell concentration changes, have not been well elucidated for even the most common model organisms (Parker, 1993; Schliess and Haüssinger, 2002).

Given the similarities in the likely damage caused by low temperature and dehydration, it has been suggested by several authors that the biochemical mechanisms enabling survival of low temperatures may simply be shared stress pathways which are also utilized under desiccation stress (Pullin, 1996; Worland and Block, 2003). There are several reasons why these views have arisen, although the degree to which these traits have co-evolved, or if the co-related responses may be considered adaptive, is not yet clear (Sinclair *et al.*, 2003b). The principal mechanism linking cold tolerance and desiccation resistance is at least partly one of physical chemistry, such that a smaller volume of fluid will freeze at lower temperatures (Salt, 1956; Worland, 1996; Denlinger and Lee, 1998; Worland, 2005). It is, therefore, no coincidence that the most desiccation- and cold-tolerant ectotherm species on the planet are also the smallest (Watanabe *et al.*, 2002; Alpert, 2006). This loss of body water also results in the concentration of molecules in solution, and can be another advantage of dehydration during cold exposure (Salt, 1956; Worland, 1996; Chown and Nicolson, 2004). As a result, cryoprotective dehydration is now recognized as an important strategy which some arthropods and several other invertebrates use to survive overwintering (Holmstrup *et al.*, 2002a; Chown and Nicolson, 2004; Bennett *et al.*, 2005). For example, in the collembolan, *Onychuirus arcticus,* loss of water to surrounding ice enables the SCP to drop from −6.5 to c. −17 °C (Worland *et al.*, 1998; Holmstrup *et al.*, 2002a).

However, simple changes in body water alone do not necessarily explain why some terrestrial arthropods show increased survival at temperatures well above their freezing point, as in freeze-intolerant species, nor does it explain enhanced survival of cold stress at temperatures above 0 °C. These can be explained by the wide array of intracellular sugar and polyhydric alcohol cryoprotectants, thermal hysteresis proteins, heat shock proteins, and membrane-bound proteins that are synthesized in response to either cold or dehydration and that, in many instances, underlie cross tolerance (see above and Koštál and Šimek, 1996; Storey and Storey, 1996; Chown and Nicolson, 2004; Bennett *et al.*, 2005). For example, in the collembolan, *Folsomia candida*, changes in total membrane phospholipid fatty acid composition during humidity acclimation is similar to those observed during cold exposure, in conjunction with the accumulation of intracellular cryoprotectants (Holmstrup *et al.*, 2002b). Moreover, an enhanced cold tolerance follows the humidity treatment (Bayley *et al.*, 2001; Holmstrup *et al.*, 2002b). In larvae of the freeze-tolerant *Pringleophaga marioni* increased tolerance of low temperature follows a desiccation pre-treatment (Sinclair and Chown, 2003), and those individuals of *Anthonomus pomorum* which survive desiccation best are those that have high trehalose contents, a pattern similar to individuals that are most tolerant of low temperature (Koštál and Šimek, 1996).

In many terrestrial arthropods, both desiccation and low temperature stimulate the production of glycerol (e.g. reviewed in Chown and Nicolson,

2004, see also Yoder *et al*., 2006). The accumulation of low molecular weight organic molecules has also been implicated in the absorption of atmospheric water for springtails (Bayley and Holmstrup, 1999) and for protection of cells against osmotic damage during extreme dehydration (Danks, 1999, 2000). In *Eurosta solidaginis* larvae during the early onset of winter, reductions in water loss rates are not correlated with changes in cold tolerance, nor are they associated with changes in haemolymph osmolality or body water content (Williams *et al*., 2004). However, a second phase of increased desiccation resistance was associated with an increase in haemolymph osmolality. Williams *et al*. (2004) speculated that interactions between cryoprotectants such as glycerol, which bind water, making it resistant to freezing and removal by dehydration, and anti-freeze proteins (or in the specific case of *E. solidaginis,* a dehydrin-like protein) act to lower the permeability of the cuticular barrier. In the flesh fly, *Sarcophaga bullata*, treatment with an exogenous glycerol dose increases both low-temperature tolerance and dehydration resistance (Yoder *et al*., 2006).

The expression of heat shock proteins in response to desiccation has also been investigated in several species (Tammariello *et al*., 1999; Bayley *et al*., 2001; Hayward *et al*., 2004a), although a relationship between cold shock and desiccation has not always been found (Goto *et al*., 1998). In *Sarcophaga crassipalpis*, which shows a complex pattern of up- and down-regulation of different heat shock proteins over the course of diapause (Hayward *et al*., 2005), heat shock protein expression in response to dehydration and rehydration has recently been carefully explored (Hayward *et al*., 2004a). In non-diapausing pupae, Hsp23 and Hsp70 are upregulated by desiccation, although the threshold for expression depends on dehydration rate. The upregulation results in a delay in eclosion, which is in keeping with previous findings that certain Hsps may interfere with the cell cycle (see Denlinger, 2002). By contrast, in diapausing pupae, which upregulate Hsp23 and Hsp70 on entry into diapause (Hayward *et al*., 2005), no Hsps were upregulated. During rehydration, both Hsp90 and Hsc70 (constitutive heat shock protein or the heat shock cognate) are upregulated in diapausing and non-diapausing pupae, and it appears that this response is very similar to the one shown following exposure to low temperatures. The role of Hsps in stabilizing both proteins and membranes clearly accounts for their significant roles during dehydration and rehydration, and it seems unlikely that they act in isolation from other cellular processes (Arispe *et al*., 2002; Tsvetkova *et al*., 2002; Hayward *et al*., 2004a).

Although much of the literature has been concerned with cross tolerance, and the identification of the similarities between responses to low temperature and dehydration, trade-offs in responses might also occur especially given modifications in lipid content and type. In *D. melanogaster,* it is well known that in response to starvation substantial changes in lipid content take place (by contrast with response to desiccation which more typically

involve changes in glycogen stores – see Gibbs *et al.*, 2003). Hence, a trade-off between starvation resistance and low-temperature tolerance was predicted by Hoffmann *et al.* (2005b). This is indeed what they found in *D. melanogaster*. Following selection for starvation resistance, low-temperature tolerance declined, although the response was sex specific. The biochemistry underlying the response remains poorly investigated, and it is not yet clear how widespread such trade-offs might be in other species. This response is also different to the one more typically investigated in the context of cold tolerance: the decline in SCPs with gut clearance or starvation (see Klok and Chown, 1998; Salin *et al.*, 2000; Chown and Nicolson, 2004).

Thus, for many terrestrial arthropod species, survival of low temperatures is based on mechanisms that are similar to those required for surviving dehydration, and in many instances, the stresses are simultaneous. Moreover, the nature of the low-temperature response might affect the extent of dehydration, which may feed back to alter the former. For example, a supercooled insect is much more likely to experience dehydration in the presence of ice than is a frozen insect (Lundheim and Zachariassen, 1993). The likelihood of dehydration might substantially influence the cold tolerance strategy that is adopted (see above and Section 6.2.2). Although little evidence exists for a direction of evolution, given the presumed date of origin of many insect taxa (see Shear and Kukalová-Peck, 1990; Labandeira and Sepkoski, 1993), and the likely conditions of the planet at the time (Stanley, 1989; Behrensmeyer *et al.*, 1992), it seems most plausible to presume that the first responses were to desiccation, and that they formed a suite of mechanisms which were subsequently honed and modified to accommodate tolerance of low temperatures.

## 7 Conclusions

We commenced this review by pointing out that humans are affecting fundamental changes to the landscape and climate of the planet, and suggesting that understanding and prediction of the consequences of these changes will require comprehension of the physiological responses of insects to their environments. This view is shared by many evolutionary physiologists (e.g. Hoffmann and Parsons, 1997; Helmuth *et al.*, 2005; Parsons, 2005), and by an increasingly wide variety of ecologists (e.g. Brown *et al.*, 2004; Owen-Smith, 2005; Wiens and Graham, 2005). In several ways, these fields, which separated in the middle of the last century, are once again beginning to be integrated (see discussions in Spicer and Gaston, 1999; Chown and Storey, 2006).

Here, we have sought to demonstrate that such integration of a variety of approaches, including models of range limits and the development of

plasticity, the assessment of environmental variability, and the exploration of responses at a wide variety of spatial and temporal scales, is of considerable value. The likely role of interactions between plasticity and stress in affecting responses to rapidly changing environments is especially significant, and has been identified as a key component missing from many assessments of the responses of organisms to environmental change (Helmuth *et al.*, 2005). Indeed, recent work has suggested that stress responses might act as a capacitor for evolution (Garland and Kelly, 2006), which might substantially alter predictions of change.

For example, the responsiveness of Hsp90 to proteins denatured by heat stress may also be the cause of the expression of phenocopies, or developmental abnormalities that resemble specific mutations and by genetic accommodation these might later be permanently expressed (Denlinger and Yocum, 1998). Hsp90 has been identified as a capacitor of morphological evolution in several species (Rutherford and Lindquist, 1998; Queitsch *et al.*, 2002), although the process is complex and may be trait specific (Milton *et al.*, 2003b). Evolution of phenotypes by genetic accommodation might also take place via changes in hormonal titres, as has been demonstrated for a colour polyphenism in *Manduca sexta* caterpillars (Suzuki and Nijhout, 2006). Such environmental perturbations might extend beyond traits that are immediately affected, and could result in changes to others such as the extent of wing venation and development, especially if alterations in hormonal titres and heat shock protein responses are involved (Roff, 1986; Marcus, 2001). Consequently, changes in mobility as well as physiological traits might evolve in response to stress, in ways that are not intuitively obvious. Certainly, in the context of rapidly changing dispersal capabilities in insects that are experiencing substantial landscape and climate change these interactions deserve closer attention (see e.g. Thomas *et al.*, 2001, but also Simmons and Thomas, 2004).

Whether the kinds of integration we have sought to promote will reveal fundamental biological laws or remain a documentation of individual responses to different conditions is a significant question. A similar question has recently occupied ecologists (see Lawton, 1999; Simberloff, 2004), and has emerged in discussions of the value of macrophysiology (Chown *et al.*, 2003, 2004b; Hodkinson, 2003). Clearly, several broad generalizations are emerging from investigations of individual and population responses to the thermal environment. For example, virtually all populations that have been examined to date show plastic responses to low-temperature treatments (see also Rako and Hoffmann, 2006). Likewise, irrespective of the level of analysis, it appears that upper lethal limits and responses to high temperature show a much narrower range of variation than do lower lethal limits and responses to low temperatures (Chown, 2001; Chown and Nicolson, 2004). However, exceptions do exist (e.g. Sinclair and Chown, 2003 for lack of responsiveness to low-temperature acclimation in *P. marioni*).

Like Simberloff (2004), we do not consider this a problem for the field, given that both understanding and prediction are essential components of the scientific endeavour, and that the two may not be related in any way (Casti, 1991). In other words, understanding of the responses of a given population or species might not result in subsequent predictive capacity, nor might prediction of the effect of a given environmental or other manipulation necessarily presuppose complete understanding of the underlying mechanisms. However, what is critical, especially in the context of the demands being placed on evolutionary physiologists by conservation biologists and ecologists, is the identification of those cases where understanding is required for prediction. It is here that insect evolutionary physiology faces its greatest challenges.

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## References

Addo-Bediako, A., Chown, S. L. and Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proc. R. Soc. Lond. B* **267**, 739–745.

Addo-Bediako, A., Chown, S. L. and Gaston, K. J. (2001). Revisiting water loss in insects: a large scale view. *J. Insect Physiol.* **47**, 1377–1388.

Addo-Bediako, A., Chown, S. L. and Gaston, K. J. (2002). Metabolic cold adaptation in insects: a large-scale perspective. *Funct. Ecol.* **16**, 332–338.

Agrawal, A. A. (2001). Phenotypic plasticity in the interaction and evolution of species. *Science* **294**, 321–326.

Alleaume-Benharira, M., Pen, I. R. and Ronce, O. (2006). Geographical patterns of adaptation within a species' range: interactions between drift and gene flow. *J. Evol. Biol.* **19**, 203–215.

Allen, A. P., Brown, J. H. and Gillooly, J. F. (2002). Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science* **297**, 1545–1548.

Alonso-Mejía, A., Arellano-Guillermo, A. and Brower, L. P. (1992). Influence of temperature, surface body moisture and height aboveground on survival of monarch butterflies overwintering in Mexico. *Biotropica* **24**, 415–419.

Alonso-Mejía, A., Rendon-Salinas, E., Montesinos-Patiño, E. and Brower, L. P. (1997). Use of lipid reserves by monarch butterflies overwintering in Mexico: implications for conservation. *Ecol. Appl.* **7**, 934–947.

Alpert, P. (2006). Constraints of tolerance: why are desiccation-tolerant organisms so small or rare? *J. Exp. Biol.* **209**, 1575–1584.

Altner, H. and Loftus, R. (1985). Ultrastructure and function of insect thermo- and hygro-receptors. *Annu. Rev. Entomol.* **30**, 273–295.

Altner, H. and Prillinger, L. (1980). Ultrastructure of invertebrate chemo-, thermo- and hygro-receptors and its functional significance. *Int. Rev. Cytol.* **67**, 69–139.

Anderson, A. R., Hoffmann, A. A. and McKechnie, S. W. (2005). Response to selection for rapid chill-coma recovery in *Drosophila melanogaster*: physiology and life history traits. *Gen. Res.* **85**, 15–22.

Anderson, J. B. and Brower, L. P. (1996). Freeze-protection of overwintering monarch butterflies in Mexico: critical role of the forest as a blanket and an umbrella. *Ecol. Entomol.* **21**, 107–116.

Andrewartha, H. G. and Birch, L. C. (1954). *The Distribution and Abundance of Animals*. Chicago: University of Chicago Press.

Angilletta, M. J., Bennett, A. F., Guderley, H., Navas, C. A., Seebacher, F. and Wilson, R. S. (2006). Coadaptation: a unifying principle in evolutionary thermal biology. *Physiol. Biochem. Zool.* **79**, 282–294.

Angilletta, M. J., Niewiarowski, P. H. and Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. *J. Thermal Biol.* **27**, 249–268.

Angilletta, M. J., Wilson, R. S., Navas, C. A. and James, R. S. (2003). Trade-offs and the evolution of thermal reaction norms. *Trends Ecol. Evol.* **18**, 234–240.

Arispe, N., Doh, M. and De Maio, A. (2002). Lipid interaction differentiates the constitutive and stress-induced heat shock proteins Hsc70 and Hsp90. *Cell Stress Chaperon* **7**, 330–338.

Ashby, P. D. (1997). Conservation of mass-specific metabolic rate among high- and low-elevation populations of the acridid grasshopper *Xanthippus corallipes*. *Physiol. Zool.* **70**, 701–711.

Ayrinhac, A., Debat, V., Gibert, P., Kister, A.-G., Legout, H., Moreteau, B., Vergilino, R. and David, J. R. (2004). Cold adaptation in geographical populations of *Drosophila melanogaster*: phenotypic plasticity is more important than genetic variability. *Funct. Ecol.* **18**, 700–706.

Bale, J. S. (1987). Insect cold hardiness: freezing and supercooling – an ecophysiological perspective. *J. Insect Physiol.* **33**, 899–908.

Bale, J. S. (1993). Classes of insect cold hardiness. *Funct. Ecol.* **7**, 751–753.

Bale, J. S. (2002). Insects and low temperatures: from molecular biology to distributions and abundance. *Philos. Trans. R. Soc. Lond. B* **357**, 849–861.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D. and Whittaker, J. B. (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Glob. Change Biol.* **8**, 1–16.

Bale, J. S., Worland, M. R. and Block, W. (2001). Effects of summer frost exposures on the cold tolerance strategy of a sub-Antarctic beetle. *J. Insect Physiol.* **47**, 1161–1167.

Bayley, M. and Holmstrup, M. (1999). Water vapour absorption in arthropods by accumulation of myoinositol and glucose. *Science* **285**, 1909–1911.

Bayley, M., Petersen, S. O., Knigge, T., Köhler, H.-R. and Holmstrup, M. (2001). Drought acclimation confers cold tolerance in the soil collembolan *Folsomia candida*. *J. Insect Physiol.* **47**, 1197–1204.

Behrensmeyer, A. K., Damuth, J. D., DiMichele, W. A., Potts, R., Sues, H.-D. and Wing, S. L. (1992). *Terrestrial Ecosystems through Time. Evolutionary Paleoecology of Terrestrial Plants and Animals*. Chicago: University of Chicago Press.

Beier, P., Van Drielen, M. and Kankam, B. O. (2002). Avifaunal collapse in West African forest fragments. *Conserv. Biol.* **16**, 1097–1111.

Bennett, A. F. and Lenski, R. E. (1997). Evolutionary adaptation to temperature. VI. Phenotypic acclimation and its evolution in *Escherichia coli*. *Evolution* **51**, 36–44.

Bennett, V. A., Sformo, T., Walters, K., Toien, O., Jeannet, K., Hochstrasser, R., Pan, Q., Serianni, A. S., Bernes, B. M. and Duman, J. G. (2005). Comparative overwintering physiology of Alaska and Indiana populations of the beetle *Cucujus clavipes* (Fabricius): roles of antifreeze proteins, polyols, dehydration and diapause. *J. Exp. Biol.* **208**, 4467–4477.

Berrigan, D. M. and Scheiner, S. M. (2004). Phenotypic plasticity. Functional and conceptual approaches. In: *Modelling the Evolution of Phenotypic Plasticity* (eds DeWitt, T. J. and Scheiner, S. M.), pp. 82–97. Oxford: Oxford University Press.

Bestelmeyer, B. T. (2000). The trade-off between thermal tolerance and behavioural dominance in a subtropical South American ant community. *J. Anim. Ecol.* **69**, 998–1009.

Bettencourt, B. R., Kim, I., Hoffmann, A. A. and Feder, M. E. (2002). Response to natural and laboratory selection at the *Drosophila HSP70* genes. *Evolution* **56**, 1796–1801.

Blackburn, T. M., Cassey, P., Duncan, R. P., Evans, K. L. and Gaston, K. J. (2004). Avian extinction and mammalian introductions on oceanic islands. *Science* **305**, 1955–1958.

Blancafort, X. and Gómez, C. (2005). Consequences of the Argentine ant, *Linepithema humile* (Mayr), invasion on pollination of *Euphorbia characias* (L.) (Euphorbiaceae). *Acta Oecol.* **28**, 49–55.

Bland, R. G., Slaney, D. P. and Weinstein, P. (1998). Antennal sensilla on cave species of Australian *Paratemnopteryx* cockroaches (Blattaria: Blattellidae). *Int. J. Ins. Morph. Embryol.* **27**, 83–93.

Block, W. (1990). Cold tolerance of insects and other arthropods. *Philos. Trans. R. Soc. Lond. B* **326**, 613–633.

Block, W. (1991). To freeze or not to freeze? Invertebrate survival of sub-zero temperatures. *Funct. Ecol.* **5**, 284–290.

Block, W. (1996). Cold or drought – the lesser of two evils for terrestrial arthropods? *Eur. J. Entomol.* **93**, 325–339.

Blows, M. W. and Hoffmann, A. A. (2005). A reassessment of genetic limits to evolutionary change. *Ecology* **86**, 1371–1384.

Blum, M. S. (1985). *Fundamentals of Insect Physiology*. New York: Wiley.

Bonan, G. B. (1999). Frost followed the plow: impacts of deforestation on the climate of the United States. *Ecol. Appl.* **9**, 1305–1315.

Bonan, G. B. (2002). *Ecological Climatology. Concepts and Applications*. Cambridge: Cambridge University Press.

Bowler, K. (2005). Acclimation, heat shock and hardening. *J. Thermal Biol.* **30**, 125–130.

Branscome, D. D., Koehler, P. G. and Oi, F. M. (2005). Influence of carbon dioxide gas on German cockroach (Dictyoptera: Blattellidae) knockdown, recovery, movement and feeding. *Physiol. Entomol.* **30**, 144–150.

Briers, R. A., Gee, J. H. R. and Geoghegan, R. (2004). Effects of the North Atlantic oscillation on growth and phenology of stream insects. *Ecography* **27**, 811–817.

Brooks, T. M., Pimm, S. L., Kapos, V. and Ravilious, C. (1999). Threat from deforestation to montane and lowland birds and mammals in insular South-east Asia. *J. Anim. Ecol.* **68**, 1061–1078.

Brooks, T. M., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., Rylands, A. B., Konstant, W. R., Flick, P., Pilgrim, J., Oldfield, S., Magin, G. and Hilton-Taylor, C. (2002). Habitat loss and extinction in the hotspots of biodiversity. *Conserv. Biol.* **16**, 909–923.

Brown, C. L., Bale, J. S. and Walters, K. F. A. (2004). Freezing induces a loss of freeze tolerance in an overwintering insect. *Proc. R. Soc. Lond. B* **271**, 1507–1511.

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. and West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.

Burton, V., Mitchell, H. K., Young, P. and Petersen, N. S. (1988). Heat shock protection against cold stress of *Drosophila melanogaster*. *Mol. Cell. Biol.* **8**, 3550–3552.

Cadet, C., Ferriere, R., Metz, J. A. J. and van Baalen, M. (2003). The evolution of dispersal under demographic stochasticity. *Am. Nat.* **162**, 427–441.

Carter, R. N. and Prince, S. D. (1981). Epidemic models used to explain biogeographical distribution limits. *Nature* **293**, 644–645.

Case, T. J. and Taper, M. L. (2000). Interspecific competition, environmental gradients, gene flow, and the coevolution of species' borders. *Am. Nat.* **155**, 583–605.

Casti, J. L. (1991). *Searching for Certainty. What Science can Know about the Future*. London: Abacus.

Catala, S. S., Maida, D. M., Caro-Riaño, H., Jaramillo, N. and Moreno, J. (2004). Changes associated with laboratory rearing in antennal sensilla patterns of *Triatoma infestans*, *Rhodnius prolixus*, and *Rhodnius pallescens* (Hemiptera, Reduviidae, Triatominae). *Mem. Inst. Oswaldo Cruz* **99**, 25–30.

Cavicchi, S., Guerra, D., La Torre, V. and Huey, R. B. (1995). Chromosonal analysis of heat-shock tolerance in *Drosophila melanogaster* evolving at different temperatures in the laboratory. *Evolution* **49**, 676–684.

Cerdá, X., Retana, J. and Manzaneda, A. (1998). The role of competition by dominants and temperature in the foraging of subordinate species in Mediterranean ant communities. *Oecologia* **117**, 404–412.

Chakir, M., Chafik, A., Moreteau, B., Gibert, P. and David, J. R. (2005). Male sterility thresholds in *Drosophila*: *D. simulans* appears more cold-adapted than its sibling *D. melanogaster*. *Genetica* **114**, 195–205.

Chamberlin, M. E. and Strange, K. (1989). Anisoosmotic cell volume regulation: a comparative view. *Am. J. Physiol.* **257**, C159–C173.

Chapuis, J. L., Boussès, P. and Barnaud, G. (1994). Alien mammals, impact and management in the French subantarctic islands. *Biol. Conserv.* **67**, 97–104.

Chase, J. M. (1996). Abiotic controls of trophic cascades in a simple grassland food chain. *Oikos* **77**, 495–506.

Chen, B., Kayukawa, T., Jiang, H., Monteiro, A., Hoshizaki, S. and Ishikawa, Y. (2005b). *DaTrypsin*, a novel clip-domain serine protease gene up-regulated during winter and summer diapauses of the onion maggot, *Delia antiqua*. *Gene* **347**, 115–123.

Chen, B., Kayukawa, T., Monteiro, A. and Ishikawa, Y. (2005a). The expression of the *HSP90* gene in response to winter and summer diapauses and thermal-stress in the onion maggot, *Delia antiqua*. *Ins. Mol. Biol.* **14**, 697–702.

Chen, C.-P. and Denlinger, D. L. (1992). Reduction of cold injury in flies using an intermittent pulse of high temperature. *Cryobiology* **29**, 138–143.

Chen, C.-P., Denlinger, D. L. and Lee, R. E. (1987). Cold-shock injury and rapid cold hardening in the flesh fly *Sarcophaga crassipalpis*. *Physiol. Zool.* **60**, 297–304.

Chen, C.-P., Lee, R. E. and Denlinger, D. L. (1990). A comparison of the responses of tropical and temperate flies (Diptera: Sarcophagidae) to cold and heat stress. *J. Comp. Physiol. B* **160**, 543–547.

Chen, C.-P., Lee, R. E. and Denlinger, D. L. (1991). Cold shock and heat shock: a comparison of the protection generated by brief pretreatment at less severe temperatures. *Physiol. Entomol.* **16**, 19–26.

Chen, C.-P. and Walker, V. K. (1994). Cold-shock and chilling tolerance in *Drosophila*. *J. Insect Physiol.* **40**, 661–669.

Chown, S. L. (1993). Desiccation resistance in six sub-Antarctic weevils (Coleoptera: Curculionidae): humidity as an abiotic factor influencing assemblage structure. *Funct. Ecol.* **7**, 318–325.

Chown, S. L. (2001). Physiological variation in insects: hierarchical levels and implications. *J. Insect Physiol.* **47**, 649–660.

Chown, S. L., Addo-Bediako, A. and Gaston, K. J. (2003). Physiological diversity: listening to the large-scale signal. *Funct. Ecol.* **17**, 562–572.

Chown, S. L. and Block, W. (1997). Comparative nutritional ecology of grass-feeding in a sub-Antarctic beetle: the impact of introduced species on *Hydromedion sparsutum* from South Georgia. *Oecologia* **111**, 216–224.

Chown, S. L. and Gaston, K. J. (1999). Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biol. Rev.* **74**, 87–120.

Chown, S. L. and Gaston, K. J. (2000). Areas, cradles and museums: the latitudinal gradient in species richness. *Trends Ecol. Evol.* **15**, 311–315.

Chown, S. L., Gaston, K. J. and Robinson, D. (2004b). Macrophysiology: large-scale patterns in physiological traits and their ecological implications. *Funct. Ecol.* **18**, 159–167.

Chown, S. L., Hull, B. and Gaston, K. J. (2005). Human impacts, energy availability, and invasion across Southern Ocean Islands. *Global Ecol. Biogeogr.* **14**, 521–528.

Chown, S. L. and Klok, C. J. (2003). Water balance characteristics respond to changes in body size in sub-Antarctic weevils. *Physiol. Biochem. Zool.* **76**, 634–643.

Chown, S. L., Klok, C. J. and McGeoch, M. A. (2004c). Weather to go out: activity of *Bothrometopus brevis* (Curculionidae) at Heard Island. *Polar Biol.* **27**, 217–221.

Chown, S. L. and Nicolson, S. W. (2004). *Insect Physiological Ecology. Mechanisms and Patterns*. Oxford: Oxford University Press.

Chown, S. L., Sinclair, B. J., Leinaas, H. P. and Gaston, K. J. (2004a). Hemispheric asymmetries in biodiversity – a serious matter for ecology. *PLoS Biol.* **2** (e406), 1701–1707.

Chown, S. L. and Smith, V. R. (1993). Climate change and the short-term impact of feral house mice at the sub-Antarctic Prince Edward Islands. *Oecologia* **96**, 508–516.

Chown, S. L. and Storey, K. B. (2006). Linking molecular physiology to ecological realities. *Physiol. Biochem. Zool.* **79**, 314–323.

Clapham, D. E. (2003). TRP channels as cellular sensors. *Nature* **426**, 517–524.

Clarke, A. (2003). Costs and consequences of evolutionary temperature adaptation. *Trends Ecol. Evol.* **18**, 573–581.

Clarke, A. (2004). Is there a universal temperature dependence of metabolism? *Funct. Ecol.* **18**, 252–256.

Clarke, A. (2006). Temperature and the metabolic theory of ecology. *Funct. Ecol.* **20**, 405–412.
Clarke, A. and Fraser, K. P. P. (2004). Why does metabolism scale with temperature? *Funct. Ecol.* **18**, 243–251.
Cohen, A. E., Gonzalez, A., Lawton, J. H., Petchey, O. L., Wildman, D. and Cohen, J. E. (1998). A novel experimental apparatus to study the impact of white noise and 1/f noise on animal populations. *Proc. R. Soc. Lond. B* **265**, 11–15.
Collinge, J. E., Hoffmann, A. A. and Mckechnie, S. W. (2006). Altitudinal patterns for latitudinally varying traits and polymorphic markers in *Drosophila melanogaster* from eastern Australia. *J. Evol. Biol.* **19**, 473–482.
Collins, S. D., Allenspach, A. L. and Lee, R. E. (1997). Ultrastructural effects of lethal freezing on brain, muscle, and Malpighian tubule from freeze-tolerant larvae of the gall fly, *Eurosta solidaginis*. *J. Insect Physiol.* **43**, 39–45.
Conrad, K. F., Woiwood, I. P. and Perry, J. N. (2003). East Atlantic teleconnection pattern and the decline of a common arctiid moth. *Global Change Biol.* **9**, 125–130.
Cossins, A. R. and Bowler, K. (1987). *Temperature Biology of Animals*. London: Chapman & Hall.
Coulson, S. J. and Bale, J. S. (1996). Supercooling and survival of the beech leaf mining weevil *Rhynchaenus fagi* L. (Coleoptera: Curculionidae). *J. Insect Physiol.* **42**, 617–623.
Coulson, S. J., Leinaas, H. P., Ims, R. A. and Søvik, G. (2000). Experimental manipulation of the winter surface ice layer: the effects on a high Arctic soil microarthropod community. *Ecography* **23**, 299–306.
Coviella, C. E. and Trumble, J. T. (1999). Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Conserv. Biol.* **13**, 700–712.
Crooks, K. R. and Soulé, M. E. (1999). Mesopredator release and avifaunal extinctions in a fragmented system. *Nature* **400**, 563–565.
Crozier, L. (2003). Winter warming facilitates range expansion: cold tolerance of the butterfly *Atalopedes campestris*. *Oecologia* **135**, 648–656.
Crozier, L. (2004). Warmer winters drive butterfly range expansion by increasing survivorship. *Ecology* **85**, 231–241.
Cunningham, S. A. (2000). Depressed pollination in habitat fragments causes low fruit set. *Proc. R. Soc. Lond. B* **267**, 1149–1152.
Danks, H. V. (1996). The wider integration of studies on insect cold hardiness. *Eur. J. Entomol.* **93**, 383–403.
Danks, H.V. (1999). Life cycles in polar arthropods–flexible or programmed? *Eur. J. Entomol.* **96**, 83–102.
Danks, H. V. (2000). Dehydration in dormant insects. *J. Insect Physiol.* **46**, 837–852.
Danks, H. V. (2003). Studying insect photoperiodism and rhythmicity: components, approaches and lessons. *Eur. J. Entomol.* **100**, 209–221.
David, J. R., Gibert, P., Gravot, E., Pétavy, G., Morin, J.-P., Karan, D. and Moreteau, B. (1997). Phenotypic plasticity and developmental temperature in *Drosophila*: analysis and significance of reaction norms of morphometrical traits. *J. Thermal Biol.* **22**, 441–451.
David, J. R., Gibert, P., Moreteau, B., Gilchrist, G. W. and Huey, R. B. (2003). The fly that came in from the cold: geographic variation of recovery time from low-temperature exposure in *Drosophila subobscura*. *Funct. Ecol.* **17**, 425–430.
Davis, A. J., Jenkinson, L. S., Lawton, J. H., Shorrocks, B. and Wood, S. (1998). Making mistakes when predicting shifts in species range in response to global warming. *Nature* **391**, 783–786.

Davis, M. B. and Shaw, R. G. (2001). Range shifts and adaptive responses to quaternary climate change. *Science* **292**, 673–679.
Deere, J. A. and Chown, S. L. (2006). Testing the beneficial acclimation hypothesis and its alternatives for locomotor performance. *American Naturalist* **168**, 630–644.
Denlinger, D. L. (1991). Relationship between cold hardiness and diapause. In: *Insects at Low Temperature* (eds Lee, R. E. and Denlinger, D. L.), pp. 174–198. New York: Chapman & Hall.
Denlinger, D. L. (2002). Regulation of diapause. *Annu. Rev. Entomol.* **47**, 93–122.
Denlinger, D. L., Giebultowicz, J. M. and Saunders, D. S. (eds) (2001). *Insect Timing: Circadian Rhythmicity to Seasonality*. Amsterdam: Elsevier.
Denlinger, D. L., Joplin, K. H., Chen, C. -P. and Lee, R. E. (1991). Cold shock and heat shock. In: *Insects at Low Temperature* (eds Lee, R. E. and Denlinger, D. L.), pp. 131–148. New York: Chapman & Hall.
Denlinger, D. L. and Lee, R. E. (1998). Physiology of cold sensitivity. In: *Temperature Sensitivity in Insects and Application in Integrated Pest Management* (eds Hallman, G. J. and Denlinger, D. L.), pp. 55–95. Boulder, CO: Westview Press.
Denlinger, D. L., Lee, R. E., Yocum, G. D. and Kukal, O. (1992). Role of chilling in the acquisition of cold tolerance and the capacitation to express stress proteins in diapausing pharate larvae of the gypsy moth, *Lymantria dispar*. *Arch. Ins. Biochem. Physiol.* **21**, 271–280.
Denlinger, D. L., Willis, J. H. and Fraenkel, G. (1972). Rates and cycles of oxygen consumption during pupal diapause in *Sarcophaga* flesh flies. *J. Insect Physiol.* **18**, 871–882.
Denlinger, D. L. and Yocum, G. D. (1998). Physiology of heat sensitivity. In: *Temperature Sensitivity in Insects and Application in Integrated Pest Management* (eds Hallman, G. J. and Denlinger, D. L.), pp. 7–53. Boulder, CO: Westview Press.
Dennill, G. B., Donnelly, D. and Chown, S. L. (1993). Expansion of host plant range of a biocontrol agent *Trichilogaster acaciaelongifoliae* released against the weed *Acacia longifolia*. *Agric. Ecosyst. Environ.* **43**, 1–10.
Dennill, G. B. and Gordon, A. J. (1990). Climate-related differences in the efficacy of the Australian gall wasp (Hymenoptera: Pteromalidae) released for the control of *Acacia longifolia* in South Africa. *Environ. Entomol.* **19**, 130–136.
DeWitt, T. J. and Scheiner, S. M. (eds) (2004). *Phenotypic Plasticity. Functional and Conceptual Approaches*. Oxford: Oxford University Press.
Didham, R. K., Tylianakis, J. M., Hutchison, M. A., Ewers, R. M. and Gemmell, N. J. (2005). Are invasive species the drivers of ecological change? *Trends Ecol. Evol.* **20**, 470–474.
Diernfellner, A. C. R., Schafmeier, T., Merrow, M. W. and Brunner, M. (2005). Molecular mechanisms of temperature sensing by the circadian clock of *Neurospora crassa*. *Genes Dev.* **19**, 1968–1973.
Donaldson, J., Nänni, I., Zachariades, C. and Kemper, J. (2002). Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. *Conserv. Biol.* **16**, 1267–1276.
Dreisig, H. (1980). Daily activity, thermoregulation and water loss in the tiger beetle *Cicindela hybrida*. *Oecologia* **44**, 376–389.
Duman, J. G. (2001). Antifreeze and ice nucleator proteins in terrestrial arthropods. *Annu. Rev. Physiol.* **63**, 327–357.
Duncan, R. P., Blackburn, T. M. and Sol, D. (2003). The ecology of bird introductions. *Annu. Rev. Ecol. Evol. Syst.* **34**, 71–98.

Dunn, R. R. (2005). Modern insect extinctions, the neglected majority. *Conserv. Biol.* **19**, 1030–1036.

Dybdahl, M. F. and Kane, S. L. (2005). Adaptation vs. phenotypic plasticity in the success of a clonal invader. *Ecology* **86**, 1592–1601.

Edney, E. B. (1977). *Water Balance in Land Arthropods*. Berlin: Springer.

Ehn, R. and Tichy, H. (1994). Hygro- and thermoreceptive tarsal organ in the spider *Cupiennius salei*. *J. Comp. Physiol. A* **174**, 345–350.

Erasmus, B. F. N., van Jaarsveld, A. S., Chown, S. L., Kshatriya, M. and Wessels, K. J. (2002). Vulnerability of South African animal taxa to climate change. *Global Change Biol.* **8**, 679–693.

Ernsting, G. (1993). Observations on life cycle and feeding ecology of two recently introduced predatory beetle species at South Georgia, sub-Antarctic. *Polar Biol.* **13**, 423–428.

Ernsting, G., Brandjes, G. J., Block, W. and Isaaks, J. A. (1999). Life-history consequences of predation for a subantarctic beetle: evaluating the contribution of direct and indirect effects. *J. Anim. Ecol.* **68**, 741–752.

Evans, K. L., Warren, P. H. and Gaston, K. J. (2005). Species–energy relationships at the macroecological scale: a review of the mechanisms. *Biol. Rev.* **79**, 1–25.

Fagan, W. F., Siemann, E., Mitter, C., Denno, R. F., Huberty, A. F., Woods, H. A. and Elser, J. J. (2002). Nitrogen in insects: implications for trophic complexity and species diversification. *Am. Nat.* **160**, 784–802.

Feder, M. E. (1987). The analysis of physiological diversity: the prospects for pattern documentation and general questions in ecological physiology. In: *New Directions in Ecological Physiology* (eds Feder, M. E., Bennett, A. F., Burggren, W. W and Huey, R. B.), pp. 38–75. Cambridge: Cambridge University Press.

Feder, M. E. (1996). Ecological and evolutionary physiology of stress proteins and the stress response: the *Drosophila melanogaster* model. In: *Animals and Temperature. Phenotypic and Evolutionary Adaptation* (eds Johnston, I. A. and Bennett, A. F.), pp. 79–102. Cambridge: Cambridge University Press.

Feder, M. E. (1999). Engineering candidate genes in studies of adaptation: the heat-shock protein Hsp70 in *Drosophila melanogaster*. *Am. Nat. Suppl*. 154, s55–s66.

Feder, M. E. and Hofmann, G. E. (1999). Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annu. Rev. Physiol.* **61**, 243–282.

Feder, M. E. and Krebs, R. A. (1998). Natural and genetic engineering of the heat-shock protein Hsp70 in *Drosophila melanogaster*: consequences for thermotolerance. *Am. Zool.* **38**, 503–517.

Feder, M. E. and Mitchell-Olds, T. (2003). Evolutionary and ecological functional genomics. *Nat. Rev. Genet.* **4**, 649–655.

Ferguson, S. H. and Messier, F. (1996). Ecological implications of a latitudinal gradient in inter-annual climatic varibility: a test using fractal and chaos theories. *Ecography* **19**, 382–392.

Fischer, K., Bauerfiend, S. S. and Fielder, K. (2006). Temperature-mediated plasticity in egg and body size in egg-size selected lines of a butterfly. *J. Thermal Biol.* **31**, 347–354.

Fischer, K., Eenhorn, E., Bot, A. N. M., Brakefield, P. M. and Zwaan, B. J. (2003). Cooler butterflies lay larger eggs: developmental plasticity versus acclimation. *Proc. R. Soc. Lond. B* **270**, 2051–2056.

Forde, S. E., Thompson, J. N. and Bohannan, B. J. M. (2004). Adaptation varies through space and time in a coevolving host–parasitoid interaction. *Nature* **431**, 841–844.

Forsman, A., Ringblom, K., Civantos, E. and Ahnesjo, J. (2002). Coevolution of color pattern and thermoregulatory behaviour in polymorphic pygmy grasshoppers *Tetrix undulata*. *Evolution* **56**, 349–360.

Frazier, M. R., Woods, H. A. and Harrison, J. F. (2001). Interactive effects of rearing temperature and oxygen on the development of *Drosophila melanogaster*. *Physiol. Biochem. Zool.* **74**, 641–650.

Frydenberg, J., Hoffmann, A. A. and Loeschcke, V. (2003). DNA sequence variation and latitudinal associations in hsp23, hsp26 and hsp27 from natural populations of *Drosophila melanogaster*. *Mol. Ecol.* **12**, 2025–2032.

Gagnon, A. S., Smoter-Tomic, K. E. and Bush, A. B. G. (2002). The El Niño southern oscillation and malaria epidemics in South America. *Intern. J. Biometeorol.* **46**, 81–99.

Gaines, S. D. and Denny, M. W. (1993). The largest, smallest, highest, lowest, longest, and shortest: extremes in ecology. *Ecology* **74**, 1677–1692.

Garland, T., Huey, R. B. and Bennett, A. F. (1991). Phylogeny and coadaptation of thermal physiology in lizards – a reanalysis. *Evolution* **45**, 1969–1975.

Garland, T. and Kelly, S. A. (2006). Phenotypic plasticity and experimental evolution. *J. Exp. Biol.* **209**, 2344–2361.

Gaston, K. J. (2003). *The Structure and Dynamics of Geographic Ranges*. Oxford: Oxford University Press.

Gaston, K. J. and Chown, S. L. (1999). Geographic range size and speciation. In: *Evolution of Biological Diversity* (eds Magurran, A. E. and May, R. M.), pp. 236–259. Oxford: Oxford University Press.

Gaston, K. J., Jones, A. G., Hänel, C. and Chown, S. L. (2003). Rates of species introduction to a remote oceanic island. *Proc. R. Soc. Lond. B* **270**, 1091–1098.

Gehring, W. J. and Wehner, R. (1995). Heat shock protein synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara desert. *Proc. Natl. Acad. Sci. USA* **92**, 2994–2998.

Ghalambor, C. K., Huey, R. B., Martin, P. R., Tewksbury, J. J. and Wang, G. (2006). Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr. Comp. Biol.* **46**, 5–17.

Gibert, P. and Huey, R. B. (2001). Chill-coma temperature in *Drosophila*: effects of developmental temperature, latitude, and phylogeny. *Physiol. Biochem. Zool.* **74**, 429–434.

Gibert, P., Huey, R. B. and Gilchrist, G. W. (2001). Locomotor performance of *Drosophila melanogaster*: interactions among developmental and adult temperatures, age, and geography. *Evolution* **55**, 205–209.

Gibbs, A. G. (1999). Laboratory selection for the comparative physiologist. *J. Exp. Biol.* **202**, 2709–2718.

Gibbs, A. G., Fukuzato, F. and Matzkin, L. M. (2003). Evolution of water conservation mechanisms in *Drosophila*. *J. Exp. Biol.* **206**, 1183–1192.

Gilchrist, G. W. (1995). Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. *Am. Nat.* **146**, 252–270.

Gilchrist, G. W. (1996). A quantitative genetic analysis of thermal sensitivity in the locomotor performance curve of *Aphidius ervi*. *Evolution* **50**, 1560–1572.

Gilchrist, G. W. and Huey, R. B. (2001). Parental and developmental temperature effects on the thermal dependence of fitness in *Drosophila melanogaster*. *Evolution* **55**, 209–214.

Gilchrist, G. W., Huey, R. B. and Partridge, L. (1997). Thermal sensitivity of *Drosophila melanogaster*: evolutionary responses of adults and eggs to laboratory natural selection at different temperatures. *Physiol. Zool.* **70**, 403–414.

Gillooly, J. F., Allen, A. P., Savage, V. M., Charnov, E. L., West, G. B. and Brown, J. H. (2006). Response to Clarke and Fraser: effects of temperature on metabolic rate. *Funct. Ecol.* **20**, 400–404.

Gillooly, J. F., Allen, A. P., West, G. B. and Brown, J. H. (2005). The rate of DNA evolution: effects of body size and temperature on the molecular clock. *Proc. Natl. Acad. Sci. USA* **102**, 140–145.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* **293**, 2248–2251.

Godde, J. and Haug, T. (1990). Analysis of the electrical responses of antennal thermo- and hygroreceptors of *Antherea* (Saturniidae, Lepidoptera) to thermal, mechanical, and electrical stimuli. *J. Comp. Physiol. A* **167**, 391–401.

Goller, F. and Esch, H. (1990). Comparative study of chill-coma temperatures and muscle potentials in insect flight muscles. *J. Exp. Biol.* **150**, 221–231.

Good, D. S. (1993). Evolution of behaviours in *Drosophila melanogaster* in high temperatures: genetic and environmental effects. *J. Insect Physiol.* **39**, 537–544.

Gordon, D. M., Moses, L., Falkovitz-Halpern, M. and Wong, E. H. (2001). Effect of weather on infestation of buildings by the invasive Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). *Am. Midl. Nat.* **146**, 321–328.

Goto, S. G. and Kimura, M. T. (1998). Heat- and cold-shock responses and temperature adaptations in subtropical and temperate species of *Drosophila*. *J. Insect Physiol.* **44**, 1233–1239.

Goto, S. G. and Kimura, M. T. (2004). Heat-shock-responsive genes are not involved in the adult diapause of *Drosophila triauraria*. *Gene* **326**, 117–122.

Goto, S. G., Yoshida, K. M. and Kimura, M. T. (1998). Accumulation of *Hsp70* mRNA under environmental stresses in diapausing and non-diapausing adults of *Drosophila triauraria*. *J. Insect Physiol.* **44**, 1009–1015.

Goulson, D. (2003). Effects of introduced bees on native ecosystems. *Annu. Rev. Ecol. Evol. Syst.* **34**, 1–26.

Grenfell, B. T., Bjørnstad, O. N. and Keppey, J. (2001). Travelling waves and spatial hierarchies in measles epidemics. *Nature* **414**, 716–723.

Gruntenko, N. E., Bownes, M., Terashima, J., Sukhanova, M. Z. and Raushenbach, I. Y. (2003a). Heat stress affects oogenesis differently in wild-type *Drosophila virilis* and a mutant with altered juvenile hormone and 20-hydroxyecdysone levels. *Ins. Mol. Biol.* **12**, 393–404.

Gruntenko, N. E., Chentsova, N. A., Andreenkova, E. V., Bownes, M., Segal, D., Adonyeva, N. V. and Rauschenbach, I. Y. (2003b). Stress response in a juvenile hormone-deficient *Drosophila melanogaster* mutant apterous (56f). *Ins. Mol. Biol.* **12**, 353–363.

Gruntenko, N. E., Wilson, T. G., Monastirioti, M. and Rauschenbach, I. Y. (2000). Stress-reactivity and juvenile hormone degradation in *Drosophila melanogaster* strains having stress-related mutations. *Ins. Bioch. Mol. Biol.* **30**, 775–783.

Guarneri, A. A., Lazzari, C., Xavier, A. A. P., Diotaiuti, L. and Lorenzo, M. G. (2003). The effect of temperature on the behaviour and development of *Triatoma brasiliensis*. *Physiol. Entomol.* **28**, 185–191.

Gutschick, V. P. and BassiriRad, H. (2003). Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. *New Phytol.* **160**, 21–42.

Hadley, N. F. (1994). *Water Relations of Terrestrial Arthropods*. San Diego, CA: Academic Press.

Hallett, T. B., Coulson, T., Pilkington, J. G., Clutton-Brock, T. H., Pemberton, J. M. and Grenfell, B. T. (2004). Why large-scale climate indices seem to predict ecological processes better than local weather. *Nature* **430**, 71–75.

Halley, J. M. (1996). Ecology, evolution and 1/*f*-noise. *Trends Ecol. Evol.* **11**, 33–37.
Hallman, G. J. and Denlinger, D. L. (1998). *Temperature Sensitivity in Insects and Application in Itegrated Pest Management*. Boulder, CO: Westview Press.
Hammack, L. (1991). Sex-pheromone communication in the screwworm, *Cochliomyia hominivorax* – ontogenetic and strain effects. *J. Chem. Ecol.* **17**, 2143–2154.
Hanc, Z. and Nedved, O. (1999). Chill injury at alternating temperatures in *Orchesella cincta* (Collembola: Entomobryidae) and *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae). *Eur. J. Entomol.* **96**, 165–168.
Hannah, L., Midgley, G. F., Lovejoy, T., Bond, W. J., Bush, M., Lovett, J. C., Scott, D. and Woodward, F. I. (2002). Conservation of biodiversity in a changing climate. *Conserv. Biol.* **16**, 264–268.
Hansen, D. M., Olesen, J. M. and Jones, C. G. (2002). Trees, birds and bees in Mauritius: exploitative competition between introduced honeybees and endemic nectarivorous birds? *J. Biogeogr.* **29**, 721–734.
Hansen, L. L., Ramløv, H. and Westh, P. (2004). Metabolic activity and water vapour absorption in the mealworm *Tenebrio molitor* L. (Coleoptera, Tenebrionidae): real-time measurements by two-channel microcalorimetry. *J. Exp. Biol.* **207**, 545–552.
Hansen, L. L., Westh, P., Wright, J. C. and Ramløv, H. (2006). Metabolic changes associated with active water vapour absorption in the mealworm *Tenebrio molitor* L. (Coleoptera, Tenebrionidae): a microcalorimetric study. *J. Insect Physiol.* **52**, 291.
Harding, K. C. and McNamara, J. M. (2002). A unifying framework for metapopulation dynamics. *Am. Nat.* **160**, 173–185.
Hargrove, J. W. (2004). Tsetse population dynamics. In: *The Trypanosomiases* (eds Maudlin, I., Holmes, P. H. and Miles, M. A.), pp. 113–138. Wallingford, CT: CABI Publishing.
Harrison, J. F. and Lighton, J. R. B. (1998). Oxygen-sensitive flight metabolism in the dragonfly *Erythemis simplicicollis*. *J. Exp. Biol.* **201**, 1739–1744.
Harshman, L. G. and Hoffmann, A. A. (2000). Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends Ecol. Evol.* **15**, 32–36.
Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S. and Samuel, M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science* **296**, 2158–2162.
Hawkins, B. A., Field, R., Cornell, H. V., Currie, D. J., Guegan, J. F., Kaufman, D. M., Kerr, J. T., Mittelbach, G. G., Oberdorf, T., O'Brien, E. M., Porter, E. E. and Turner, J. R. G. (2003). Energy, water, and broad-scale geographic patterns of species richness. *Ecology* **84**, 3105–3117.
Hayward, S. A. L., Bale, J. S., Worland, M. R. and Convey, P. (2001). Influence of temperature on the hygropreference of the Collembolan, *Cryptopygus antarcticus*, and the mite, *Alaskozetes antarcticus* from the maritime Antarctic. *J. Insect Physiol.* **47**, 11–18.
Hayward, S. A. L., Pavlides, S. C., Tammariello, S. P., Rinehart, J. P. and Denlinger, D. L. (2005). Temporal expression patterns of diapause-associated genes in flesh fly pupae from the onset of diapause through post-diapause quiescence. *J. Insect Physiol.* **51**, 631–640.
Hayward, S. A. L., Rinehart, J. P. and Denlinger, D. L. (2004a). Desiccation and rehydration elicit distinct heat shock protein transcript responses in flesh fly pupae. *J. Exp. Biol.* **207**, 963–971.
Hayward, S. A. L., Worland, M. R., Bale, J. S. and Convey, P. (2000). Temperature and the hygropreference of the Arctic Collembolan *Onychiurus arcticus* and the mite *Lauroppia translamellata*. *Physiol. Entomol.* **25**, 266–272.

Hayward, S. A. L., Worland, M. R., Convey, P. and Bale, J. S. (2004b). Habitat moisture availability and the local distribution of the Antarctic Collembola *Cryptopygus antarcticus* and *Frisea grisea*. *Soil Biol. Biochem.* **36**, 927–934.
Hazel, W., Smock, R. and Lively, C. M. (2004). The ecological genetics of conditional strategists. *Am. Nat.* **163**, 888–900.
Helmuth, B., Kingsolver, J. G. and Carrington, E. (2005). Biophysics, physiological ecology, and climate change: does mechanism matter? *Annu. Rev. Physiol.* **67**, 177–201.
Herford, G. M. (1938). Tracheal pulsation in the flea. *J. Exp. Biol.* **15**, 327–338.
Herrera, C. M. (1997). Thermal biology and foraging responses of insect pollinators to the forest floor irradiance mosaic. *Oikos* **78**, 601–611.
Hess, E. and Loftus, R. (1984). Warm and cold receptors of two sensilla on the foreleg tarsi of the tropical bont tick *Amblyomma variegatum*. *J. Comp. Physiol. A* **155**, 187–195.
Hill, J. K., Hughes, C. L., Dytham, C. and Searle, J. B. (2006). Genetic diversity in butterflies: interactive effects of habitat fragmentation and climate-driven range expansion. *Biol. Lett.* **2**, 152–154.
Hill, J. K., Thomas, C. D. and Huntley, B. (1999). Climate and habitat availability determine 20th century changes in a butterfly's range margin. *Proc. R. Soc. Lond. B* **266**, 1197–1206.
Hochachka, P. W. and Somero, G. N. (2002). *Biochemical Adaptation. Mechanisms and Processes in Physiological Evolution*. New York: Oxford University Press.
Hódar, J. A., Castro, J. and Zamora, R. (2003). Pine processionary caterpillar *Thaumetopoea pityocampa* as a new threat for relict Mediterranean Scots pine forests under climatic warming. *Biol. Conserv.* **110**, 123–129.
Hodkinson, I. D. (2003). Metabolic cold adaptation in arthropods: a smaller-scale perspective. *Funct. Ecol.* **17**, 562–567.
Hodkinson, I. D., Coulson, S. J., Webb, N. R., Block, W., Strathdee, A. T., Bale, J. S. and Worland, M. R. (1996). Temperature and the biomass of flying midges (Diptera: Chironomidae) in the high Arctic. *Oikos* **75**, 241–248.
Hoekstra, H. E., Hoekstra, J. M., Berrigan, D., Vignieri, S. N., Hoang, A., Hill, C. E. and Beerli, P. (2001). Strength and tempo of directional selection in the wild. *Proc. Natl. Acad. Sci. USA* **98**, 9157–9160.
Hoffmann, A. A. (1995). Acclimation: increasing survival at a cost. *Trends Ecol. Evol.* **10**, 1–2.
Hoffmann, A. A. and Blows, M. W. (1994). Species borders: ecological and evolutionary perspectives. *Trends Ecol. Evol.* **9**, 223–227.
Hoffmann, A. A., Hallas, R. J., Anderson, A. R. and Telonis-Scott, M. (2005b). Evidence for a robust sex-specific trade-off between cold resistance and starvation resistance in *Drosophila melanogaster*. *J. Evol. Biol.* **18**, 804–810.
Hoffmann, A. A., Hallas, R. J., Dean, J. A. and Schiffer, M. (2003a). Low potential for climatic stress adaptation in a rainforest *Drosophila* species. *Science* **301**, 100–102.
Hoffmann, A. A., Hallas, R., Sinclair, C. and Partridge, L. (2001). Rapid loss of stress resistance in *Drosophila melanogaster* under adaptation to laboratory culture. *Evolution* **55**, 436–438.
Hoffmann, A. A. and Hewa-Kapuge, S. (2000). Acclimation for heat resistance in *Trichogramma nr. brassicae*: can it occur without costs? *Funct. Ecol.* **14**, 55–60.
Hoffmann, A. A. and Parsons, P. A. (1997). *Extreme Environmental Change and Evolution*. Cambridge: Cambridge University Press.
Hoffmann, A. A., Scott, M., Partridge, L. and Hallas, R. (2003c). Overwintering in *Drosophila melanogaster*: outdoor field cage experiments on clinal and laboratory selected populations help to elucidate traits under selection. *J. Evol. Biol.* **16**, 614–623.

Hoffmann, A. A., Shirriffs, J. and Scott, M. (2005a). Relative importance of plastic versus genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Funct. Ecol.* **19**, 222–227.

Hoffmann, A. A., Sørensen, J. G. and Loeschcke, V. (2003b). Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Thermal Biol.* **28**, 175–216.

Hoffmann, A. A. and Watson, M. (1993). Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. *Am. Nat.* **142**, S93–S113.

Holmgren, M., Scheffer, M., Ezcurra, E., Gutiérrez, J. R. and Mohren, G. M. J. (2001). El Niño effects on the dynamics of terrestrial ecosystems. *Trends Ecol. Evol.* **16**, 89–94.

Holmstrup, M., Bayley, M. and Ramløv, H. (2002a). Supercool or dehydrate? An experimental analysis of overwintering strategies in small permeable artic invertebrates. *Proc. Natl. Acad. Sci. USA* **99**, 5716–5720.

Holmstrup, M., Hedlund, K. and Boriss, H. (2002b). Drought acclimation and lipid composition in *Folsomia candida*: implications for cold shock, heat shock and acute desiccation stress. *J. Insect Physiol.* **48**, 961–970.

Holt, R. D., Barfield, M. and Gomulkiewicz, R. (2004a). Temporal variation can facilitate niche evolution in harsh sink environments. *Am. Nat.* **164**, 187–200.

Holt, R. D. and Keitt, T. H. (2000). Alternative causes for range limits: a metapopulation perspective. *Ecol. Lett.* **3**, 41–47.

Holt, R. D., Knight, T. M. and Barfield, M. (2004b). Allee effects, immigration, and the evolution of species' niches. *Am. Nat.* **163**, 253–262.

Holway, D. A. and Suarez, A. V. (2006). Homogenization of ant communities in mediterranean California: the effects of urbanization and invasion. *Biol. Conserv.* **127**, 319–326.

Hosler, J. S., Burns, J. E. and Esch, H. E. (2000). Flight muscle resting potential and species-specific differences in chill-coma. *J. Insect Physiol.* **46**, 621–627.

Huestis, D. L. and Marshall, J. L. (2006). Interaction between maternal effects and temperature affects diapause occurrence in the cricket *Allonemobius socialis*. *Oecologia* **146**, 513–520.

Huey, R. B. and Berrigan, D. (1996). Testing evolutionary hypotheses of acclimation. In: *Animals and Temperature. Phenotypic and Evolutionary Adaptation* (eds Johnston, I. A. and Bennett, A. F.), pp. 205–237. Cambridge: Cambridge University Press.

Huey, R. B., Berrigan, D., Gilchrist, G. W. and Herron, J. C. (1999). Testing the adaptive significance of acclimation: a strong inference approach. *Am. Zool.* **39**, 323–336.

Huey, R. B., Hertz, P. E. and Sinervo, B. (2003). Behavioral drive versus behavioral inertia in evolution: a null model approach. *Am. Nat.* **161**, 357–366.

Huey, R. B. and Kingsolver, J. G. (1993). Evolution of resistance to high temperature in ectotherms. *Am. Nat.* **142**, S21–S46.

Huey, R. B., Partridge, L. and Fowler, K. (1991). Thermal sensitivity of *Drosophila melanogaster* responds rapidly to laboratory natural selection. *Evolution* **45**, 751–756.

Hulbert, A. J. (2003). Life, death and membrane bilayers. *J. Exp. Biol.* **206**, 2303–2311.

Hunger, T. and Steinbrecht, R. A. (1998). Functional morphology of a double-walled multiporous olfactory sensillum: the sensillum coeloconicum of *Bombyx mori* (Insecta, Lepidoptera). *Tissue and Cell* **30**, 14–29.

Huntley, B., Green, R. E., Collingham, Y. C., Hill, J. K., Willis, S. G., Bartlein, P. J., Cramer, W., Hagemeijer, W. J. M. and Thomas, C. J. (2004). The performance of models relating species geographical distributions to climate is independent of trophic level. *Ecol. Lett.* **7**, 417–426.

Huyton, P. M. and Brady, J. (1975). Some effects of light and heat on feeding and resting behavior of tsetse flies, *Glossina morsitans* Westwood. *J. Entomol. A* **50**, 23–30.

Inoshita, T. and Tanimura, T. (2006). Cellular identification of water gustatory receptor neurons and their central projection pattern in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **103**, 1094–1099.

Irwin, J. T., Bennet, V. A. and Lee, R. E. (2001). Diapause development in frozen larvae of the goldenrod gall fly, *Eurosta solidaginis* Fitch (Diptera: Tephritidae). *J. Comp. Physiol. B* **171**, 181–188.

Irwin, J. T. and Lee, R. E. (2000). Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae). *J. Insect Physiol.* **46**, 655–661.

Irwin, J. T. and Lee, R. E. (2002). Energy and water conservation in frozen vs. supercooled larvae of the goldenrod gall fly, *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae). *J. Exp. Zool.* **292**, 345–350.

Irwin, J. T. and Lee, R. E. (2003). Cold winter microenvironments conserve energy and improve overwintering survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis*. *Oikos* **100**, 71–78.

Izumi, Y., Sonoda, S., Yoshida, H. and Tsumuki, H. (2005). Identification of tissues showing the lowest tolerance to freezing in larvae of the rice stem borer, *Chilo suppressalis*. *Physiol. Entomol.* **30**, 324–331.

Janiszewski, J. (1986). The effect of temperature changes on the spontaneous activity in the neural ganglia of the cockroach, *Periplaneta americana*. *J. Therm. Biol.* **11**, 191–197.

Jeffree, C. E. and Jeffree, E. P. (1996). Redistribution of the potential geographical ranges of Mistletoe and Colorado Beetle in Europe in response to the temperature component of climate change. *Funct. Ecol.* **10**, 562–577.

Jenkins, N. L. and Hoffmann, A. A. (1999). Limits to the southern border of *Drosophila serrata*: cold resistance, heritable variation, and trade-offs. *Evolution* **53**, 1823–1834.

Jenouvrier, S., Weimerskirch, H., Barbraud, C., Park, Y. H. and Cazelles, B. (2005). Evidence of a shift in the cyclicity of Antarctic seabird dynamics linked to climate. *Proc. R. Soc. Lond. B* **272**, 887–895.

Jian, F., Jayas, D. S. and White, N. D. G. (2005). Movement and distribution of adult *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) in stored wheat in response to temperature gradients, dockage, and moisture differences. *J. Stored Prod. Res.* **41**, 401–422.

Jian, F. J., Jayas, D. S., White, N. D. G. and Muir, W. E. (2002). Temperature and geotaxis preference by *Cryptolestes ferrugineus* (Coleoptera : Laemophloeidae) adults in response to 5 degrees C/m temperature gradients at optimum and hot temperatures in stored wheat and their mortality at high temperature. *Environ. Entomol.* **31**, 816–826.

Jing, X. H. and Kang, L. (2003). Geographical variation in egg cold hardiness: a study on the adaptation strategies of the migratory locust *Locusta migratoria* L. *Ecol. Entomol.* **28**, 151–158.

Joanisse, D. R. and Storey, K. B. (1994a). Enzyme activity profiles in an overwintering population of freeze-tolerant larvae of the gall fly, *Eurosta solidaginis*. *J. Comp. Physiol. B* **164**, 247–255.

Joanisse, D. R. and Storey, K. B. (1994b). Mitochondrial enzymes during overwintering in two species of cold-hardy gall insects. *Insect Biochem. Mol. Biol.* **24**, 145–150.

Joanisse, D. R. and Storey, K. B. (1996). Oxidative stress and antioxidants in overwintering larvae of cold-hardy goldenrod gall insects. *J. Exp. Biol.* **199**, 1483–1491.

Joanisse, D. R. and Storey, K. B. (1998). Oxidative stress and antioxidants in stress and recovery of cold-hardy insects. *Ins. Biochem. Mol. Biol.* **28**, 23–30.

Jones, B. M. (1950). The sensory physiology of the harvest mite *Trombicula autumnalis* Shaw. *J. Exp. Biol.* **27**, 461–494.

Joos, B., Lighton, J. R. B., Harrison, J. F., Suarez, R. K. and Roberts, S. P. (1997). Effects of ambient oxygen tension on flight performance, metabolism, and water loss of the honeybee. *Physiol. Zool.* **70**, 167–174.

Joplin, K. H., Yocum, G. D. and Denlinger, D. L. (1990). Cold shock elicits expression of heat shock proteins in the flesh fly, *Sarcophaga crassipalpis*. *J. Insect Physiol.* **36**, 825–834.

Jørgensen, K. T., Sørensen, J. G. and Bundgaard, J. (2006). Heat tolerance and the effect of mild heat stress on reproductive characters in *Drosophila buzzatii* males. *J. Thermal Biol.* **31**, 280–286.

Karan, D. and David, J. R. (2000). Cold tolerance in *Drosophila*: adaptive variations revealed by the analysis of starvation survival reaction norms. *J. Thermal Biol.* **25**, 345–351.

Karnosky, D. F., Zak, D. R., Pregitzer, K. S., Awmack, C. S., Bockheim, J. G., Dickson, R. E., Hendrey, G. R., Host, G. E., King, J. S., Kopper, B. J., Kruger, E. L., Kubiske, M. E., Lindroth, R. L., Mattson, W. J., McDonald, E. P., Noormets, A., Oksanen, E., Parsons, W. F. J., Percy, K. E., Podila, G. K., Riemenschneider, D. E., Sharma, P., Thakur, R., Sôber, A., Sôber, J., Jones, W. S., Anttonen, S., Vapaavuori, E., Mankovska, B., Heilman, W. and Isebrands, J. G. (2003). Tropospheric O3 moderates responses of temperate hardwood forests to elevated CO2: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Funct. Ecol.* **17**, 289–304.

Karunanithi, S., Barclay, J. W., Brown, I. R., Robertson, R. M. and Atwood, H. L. (1999). Neuroprotection at *Drosophila* synapses conferred by prior heat shock. *J. Neurosci.* **19**, 4360–4369.

Karunanithi, S., Barclay, J. W., Brown, I. R., Robertson, R. M. and Atwood, H. L. (2002). Enhancement of presynaptic performance in transgenic *Drosophila* overexpressing heat shock protein HSP70. *Synapse* **44**, 8–14.

Kelty, J. D., Killian, K. A. and Lee, R. E. (1996). Cold shock and rapid cold-hardening of pharate adult flesh flies (*Sarcophaga crassipalpis*): effects on behaviour and neuromuscular function following eclosion. *Physiol. Entomol.* **21**, 283–288.

Kelty, J. D. and Lee, R. E. (1999). Induction of rapid cold hardening by cooling at ecologically relevant rates in *Drosophila melanogaster*. *J. Insect Physiol.* **45**, 719–726.

Kelty, J. D. and Lee, R. E. (2001). Rapid cold-hardening of *Drosophila melanogaster* (Diptera: Drosophilidae) during ecologically based thermoperiodic cycles. *J. Exp. Biol.* **204**, 1659–1666.

Kevan, P. G. (1975). Sun-tracking solar furnaces in High Arctic flowers: significance for pollination and insects. *Science* **189**, 723–726.

Kimura, M. T. (2004). Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia* **140**, 442–449.

Kingsolver, J. G. (1989). Weather and the population dynamics of insects: integrating physiological and population ecology. *Physiol. Zool.* **62**, 314–334.

Kingsolver, J. G., Gomulkiewicz, R. and Carter, P. A. (2001). Variation, selection and evolution of function-valued traits. *Genetica* **112**, 87–104.
Kingsolver, J. G. and Huey, R. B. (1998). Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. *Am. Zool.* **38**, 545–560.
Kirkpatrick, M. and Barton, N. H. (1997). Evolution of a species' range. *Am. Nat.* **150**, 1–23.
Klok, C. J. and Chown, S. L. (1998). Interactions between desiccation resistance, host–plant contact and the thermal biology of a leaf-dwelling sub-antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). *J. Insect Physiol.* **44**, 615–628.
Klok, C. J. and Chown, S. L. (2003). Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biol. J. Linn. Soc.* **78**, 401–414.
Klok, C. J., Sinclair, B. J. and Chown, S. L. (2004). Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *J. Exp. Biol.* **207**, 2361–2370.
Klose, M. K., Armstrong, G. and Robertson, R. M. (2004). A role for the cytoskeleton in heat-shock-mediated thermoprotection of locust neuromuscular junctions. *Neurobiology* **60**, 453–462.
Klose, M. K. and Robertson, R. M. (2004). Stress-induced thermoprotection of neuromuscular transmission. *Integr. Comp. Biol.* **44**, 14–20.
Klvana, I., Berteaux, D. and Cazelles, B. (2004). Porcupine feeding scars and climatic data show ecosystem effects of the solar cycle. *Am. Nat.* **164**, 283–297.
Knickerbocker, D. L. and Lutz, P. L. (2001). Slow ATP loss and the defense of ion homeostasis in the anoxic frog brain. *J. Exp. Biol.* **204**, 3547–3551.
Koh, L. P., Dunn, R. R., Sodhi, N. S., Colwell, R. K., Proctor, H. C. and Smith, V. S. (2004). Species coextinctions and the biodiversity crisis. *Science* **305**, 1632–1634.
Korsloot, A., van Gestel, C. and van Straalen, N. (2004). *Environmental Stress and Cellular Response in Arthropods*. Boca Raton, FL: CRC Press.
Koštál, V. (2006). Eco-physiological phases of insect diapause. *J. Insect Physiol.* **52**, 113.
Koštál, V. and Šimek, P. (1996). Biochemistry and physiology of aestivo-hibernation in the adult apple blossom weevil, *Anthonomus pomorum* (Coleoptera: Curculionidae). *J. Insect Physiol.* **42**, 727–733.
Koštál, V., Slachta, M. and Šimek, P. (2001). Cryoprotective role of polyols independent of the increase in supercooling capacity in diapausing adults of *Pyrrhocoris apterus* (Heteroptera: Insecta). *Comp. Biochem. Physiol. B* **130**, 365–374.
Koštál, V., Vambera, J. and Bastl, J. (2004). On the nature of pre-freeze mortality in insects: water balance, ion homeostasis and energy charge in the adults of *Pyrrhocoris apterus*. *J. Exp. Biol.* **207**, 1509–1521.
Kozłowski, J., Czarnoleski, M. and Dańko, M. (2004). Can optimal resource allocation models explain why ectotherms grow larger in cold? *Integr. Comp. Biol.* **44**, 480–493.
Kozłowski, J., Konarzewski, M. and Gawelczyk, A. T. (2003). Cell size as a link between noncoding DNA and metabolic rate scaling. *Proc. Natl. Acad. Sci. USA* **100**, 14080–14085.
Krebs, R. A. and Feder, M. E. (1997). Natural variation in the expression of the heat-shock protein Hsp70 in a population of *Drosophila melanogaster* and its correlation with tolerance of ecologically relevant thermal stress. *Evolution* **51**, 173–179.
Krebs, R. A. and Loeschcke, V. (1994). Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster*. *Funct. Ecol.* **8**, 730–737.

Krebs, R. A. and Loeschcke, V. (1995). Resistance to thermal stress in adult *Drosophila buzzatii*: acclimation and variation among populations. *Biol. J. Linn. Soc.* **56**, 505–515.

Krebs, R. A. and Loeschcke, V. (1996). Acclimation and selection for increased resistance to thermal stress in *Drosophila buzzatii*. *Genetics* **142**, 471–479.

Krebs, R. A. and Loeschcke, V. (1997). Estimating heritability in a threshold trait: heat-shock tolerance in *Drosophila buzzatii*. *Heredity* **79**, 252–259.

Krebs, R. A., Roberts, S. P., Bettencourt, B. R. and Feder, M. E. (2001). Changes in thermotolerance and Hsp70 expression with domestication in *Drosophila melanogaster*. *J. Evol. Biol.* **14**, 75–82.

Krebs, R. A. and Thompson, K. A. (2005). A genetic analysis of variation for the ability to fly after exposure to thermal stress in *Drosphila mojavensis*. *J. Thermal Biol.* **30**, 335–342.

Kregel, K. C. (2002). Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *J. Appl. Physiol.* **92**, 2177–2186.

Krishnan, N. and Kodrik, D. (2006). Antioxidant enzymes in *Spodoptera littoralis* (Boisduval): are they enhanced to protect gut tissues during oxidative stress? *J. Insect Physiol.* **52**, 11–20.

Kristiansen, E. and Zachariassen, K. E. (2001). Effect of freezing on the transmembrane distribution of ions in freeze-tolerant larvae of the wood fly *Xylophagus cinctus* (Diptera, Xylophagidae). *J. Insect Physiol.* **47**, 585–592.

Kuhrt, U., Samietz, J. and Dorn, S. (2006). Thermal response in adult codling moth. *Physiol. Entomol.* **31**, 80–88.

Kukal, O. and Duman, J. G. (1989). Switch in the overwintering strategy of two insect species and latitudinal differences in cold hardiness. *Can. J. Zool.* **67**, 825–827.

Kukal, O., Duman, J. G. and Serianni, A. S. (1989). Cold-induced mitochondrial degradation and cryoprotectant synthesis in freeze-tolerant arctic caterpillars. *J. Comp. Physiol.* **158**, 661–671.

Kutz, S. J., Hoberg, E. P., Polley, L. and Jenkins, E. J. (2005). Global warming is changing the dynamics of Arctic host–parasite systems. *Proc. R. Soc. Lond. B* **272**, 2571–2576.

Labandeira, C. C. and Sepkoski, J. J. (1993). Insect diversity in the fossil record. *Science* **261**, 310–315.

Lactin, D. J. and Johnson, D. L. (1996). Behavioural optimization of body temperature by nymphal grasshoppers (*Melanoplus sanguinipes*, Orthoptera: Acrididae) in temperature gradients established using incandescent bulbs. *J. Therm. Biol.* **21**, 231–238.

Landais, I., Pommet, J.-M., Mita, K., Nohata, J., Gimenez, S., Fournier, P., Devauchelle, G., Duonor-Cerutti, M. and Ogliastro, M. (2001). Characterization of the cDNA encoding the 90 kDA heat-shock protein in the Lepidoptera *Bombyx mori* and *Spodoptera frugiperda*. *Gene* **271**, 223–231.

Lang, F., Busch, G. L., Ritter, M., Völkl, H., Waldegger, S., Gulbins, E. and Häussinger, D. (1998). Functional significance of cell volume regulatory mechanisms. *Physiol. Rev.* **78**, 247–306.

Lansing, E., Justesen, J. and Loeschcke, V. (2000). Variation in the expression of Hsp70, the major heat-shock protein, and thermotolerance in larval and adult selection lines in *Drosophila melanogaster*. *J. Thermal Biol.* **25**, 443–450.

Larsen, K. J. and Lee, R. E. (1994). Cold tolerance including rapid cold-hardening and inoculative freezing of fall migrant monarch butterflies in Ohio. *J. Insect Physiol.* **40**, 859–864.

Lawton, J. H. (1988). More time means more variation. *Nature* **344**, 563.

Lawton, J. H. (1992). There are not 10 million kinds of population dynamics. *Oikos* **63**, 337–338.
Lawton, J. H. (1999). Are there general laws in ecology? *Oikos* **84**, 177–192.
Lazzari, C. R. (1991). Temperature preference in *Triatoma infestans* (Hemiptera: Reduviidae). *Bull. Entonol. Res.* **81**, 273–276.
Leather, S. R., Walters, K. F. A. and Bale, J. S. (1993). *The Ecology of Insect Overwintering*. Cambridge: Cambridge University Press.
Lee, R. E., Chen, C.-P. and Denlinger, D. L. (1987a). A rapid cold-hardening process in insects. *Science* **238**, 1415–1417.
Lee, R. E., Chen, C., Meacham, M. H. and Denlinger, D. L. (1987b). Ontogenetic patterns of cold-hardiness and glycerol production in *Sarcophaga crassipalpis*. *J. Insect Physiol.* **33**, 587–592.
Lee, R. E. and Denlinger, D. L. (1985). Cold tolerance in diapausing and non-diapausing stages of the flesh fly, *Sarcophaga crassipalpis*. *Physiol. Entomol.* **10**, 309–315.
Lee, R. E., Dommel, R. A., Joplin, K. H. and Denlinger, D. L. (1995). Cryobiology of the freeze-tolerant gall fly *Eurosta solidaginis*: overwintering energetics and heat shock proteins. *Climate Res.* **5**, 61–67.
Lee, R. E., Elnitsky, M. A., Rinehart, J. P., Hayward, S. A. L., Sandro, L. H. and Denlinger, D. L. (2006). Rapid cold-hardening increases the freezing tolerance of the Antarctic midge *Belgica antarctica*. *J. Exp. Biol.* **209**, 399–406.
Lee, R. E., McGrath, J. J., Morason, R. T. and Taddeo, R. M. (1993). Survival of intracellular freezing, lipid coalescence and osmotic fragility in fat body cells of the freeze-tolerant gall fly *Eurosta solidaginis*. *J. Insect Physiol.* **39**, 445–450.
Lees, A. D. (1948). The sensory physiology of the sheep tick *Ixodes ricinus* L. *J. Exp. Biol.* **25**, 145–207.
Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends Ecol. Evol.* **17**, 183–189.
Lerman, D. N. and Feder, M. E. (2001). Laboratory selection at different temperatures modifies heat-shock transcription factor (HSF) activation in *Drosophila melanogaster*. *J. Exp. Biol.* **204**, 315–323.
Leroi, A. M., Bennett, A. F. and Lenski, R. E. (1994). Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proc. Natl. Acad. Sci. USA* **91**, 1917–1921.
Levins, R. (1968). *Evolution in Changing Environments*. Princeton: Princeton University Press.
Liu, L., Yermolaieva, O., Johnson, W. A., Abboud, F. M. and Welsh, M. J. (2003). Identification and function of thermosensory neurons in *Drosophila* larvae. *Nat. Neurosci.* **6**, 267–273.
Lively, C. M. (1986). Canalization versus developmental conversion in a spatially variable environment. *Am. Nat.* **128**, 561–572.
Loeschke, V. and Hoffmann, A. A. (2002). The detrimental acclimation hypothesis. *Trends Ecol. Evol.* **17**, 407–408.
Loeschcke, V., Krebs, R. A., Dahlgaard, J. and Michalak, P. (1997). High-temperature stress and the evolution of thermal resistance in *Drosophila*. In: *Environmental Stress, Adaptation and Evolution* (eds Bijlsma, R. and Loeschcke, V.), pp. 175–190. Basel: Birkhäuser.
Loeschcke, V. and Sørensen, J. G. (2005). Acclimation, heat shock and hardening – a response from evolutionary biology. *J. Thermal Biol.* **30**, 255–257.
Loftus, R. (1968). The response of the antennal cold receptor of *Periplaneta americana* to rapid temperature changes and to steady temperature. *Z. Vergl. Physiol.* **52**, 413–455.

Logue, J. A., de Vries, A. L., Fodor, E. and Cossins, A. R. (2000). Lipid compositional correlates of temperature-adaptive interspesific differences in membrane physical structure. *J. Exp. Biol.* **203**, 2105–2115.

Lorenzo, M. G. and Lazzari, C. R. (1999). Temperature and relative humidity affect the selection of shelters by *Triatoma infestans*, vector of chagas disease. *Acta Trop.* **72**, 241–249.

Lundheim, R. and Zachariassen, K. E. (1993). Water balance of over-wintering beetles in relation to strategies for cold tolerance. *J. Comp. Physiol. B* **163**, 1–4.

Lynch, M. and Gabriel, W. (1987). Environmental tolerance. *Am. Nat.* **129**, 283–303.

Macdonald, S. S., Rako, L., Batterham, P. and Hoffmann, A. A. (2004). Dissecting chill coma recovery as a measure of cold resistansb: evidence for a biphasic response in *Drosophila melanogaster*. *J. Insect Physiol.* **50**, 695–700.

Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M. and Bazzaz, F. A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol. Appl.* **10**, 689–710.

Mahroof, R., Zhu, K. Y., Neven, L., Subramanyamm, B. and Bai, J. (2005). Expression patterns of three heat shock protein 70 genes among developmental stages of the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Comp. Biochem. Physiol. A* **141**, 247–256.

Maisonhaute, C., Chihrane, J. and Laugé, G. (1999). Induction of thermotolerance in *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae). *Environ. Entomol.* **28**, 116–122.

Makarieva, A. M., Gorshkov, V. G., Li, B. L. and Chown, S. L. (2006). Size- and temperature-independence of minimum life-supporting metabolic rates. *Funct. Ecol.* **20**, 83–96.

Marcus, J. M. (2001). The development and evolution of crossveins in insect wings. *J. Anat.* **199**, 211–216.

Matos, M., Rose, M. R., Pité, M. T. R., Rego, C. and Avelar, T. (2000). Adaptation to the laboratory environment in *Drosophila subobscura*. *J. Evol. Biol.* **13**, 9–19.

May, M. L. (1979). Insect thermoregulation. *Annu. Rev. Entomol.* **24**, 313–349.

McColl, G., Hoffmann, A. A. and McKechnie, S. W. (1996). Response of two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*. *Genetics* **143**, 1615–1627.

McDonald, J. R., Bale, J. S. and Walters, K. F. A. (1997). Rapid cold hardening in the western flower thrips *Frankliniella occidentalis*. *J. Insect Physiol.* **43**, 759–766.

McDonald, J. R., Head, J., Bale, J. S. and Walters, K. F. A. (2000). Cold tolerance, overwintering and establishment potential of *Thrips palmi*. *Physiol. Entomol.* **25**, 159–166.

McGeoch, M. A. (1998). The selection, testing and application of terrestrial insects as bioindicators. *Biol. Rev.* **73**, 181–201.

McGeoch, M. A. (in press). Insects and bioindication: theory and progress. In: *Insect Conservation Biology* (eds Stewart, A. J. A, Lewis, O. T. and New, T. R.), London: CABI Publishing.

McGeoch, M. A. and Wossler, T. C. (2000). Range expansion and success of the weed biocontrol agent *Trichilogaster acaciaelongifoliae* (Froggatt) (Hymenoptera: Pteromalidae) in South Africa. *Afr. Entomol.* **8**, 273–280.

McKechnie, A. E., Freckleton, R. P. and Jetz, W. (2006). Phenotypic plasticity in the scaling of avian basal metabolic rate. *Proc. R. Soc. Lond. B* **273**, 931–937.

McNab, B. K. (2002). *The Physiological Ecology of Vertebrates: A View from Energetics*. Ithaca, NY: Cornell University Press.

Mélice, J.-L., Lutjeharms, J. R. E., Rouault, M. and Ansorge, I. J. (2003). Sea-surface temperatures at the sub-Antarctic islands Marion and Gough during the past 50 years. *S. Afr. J. Sci.* **99**, 363–366.

Merivee, E., Vanatoa, A., Luik, A., Rahi, M., Sammelselg, V. and Ploomi, A. (2003). Electrophysiological identification of cold receptors on the antennae of the ground beetle *Pterostichus aethiops*. *Physiol. Entomol.* **28**, 88–96.

Messenger, P. S. (1959). Bioclimatic studies with insects. *Annu. Rev. Entomol.* **4**, 183–206.

Michaud, M. R. and Denlinger, D. L. (2005). Molecular modalities of insect cold survival: current understanding and future trends. In: *Animals and Environments* (eds Morris, S. and Vosloo, A.), pp. 32–46. Amsterdam: Elsevier International Congress Series 1275.

Millennium Ecosystem Assessment (2005). *Ecosystems and Human Well-Being: Biodiversity Synthesis*. Washington, DC: World Resources Institute.

Miller, L. K. (1978). Freezing tolerance in relation to cooling rate in an adult insect. *Cryobiology* **15**, 345–349.

Milton, C. C., Huynh, B., Batterham, P., Rutherford, S. L. and Hoffmann, A. A. (2003b). Quantitative trait symmetry independent of Hsp90 buffering: distinct modes of genetic canalization and developmental stability. *Proc. Natl. Acad. Sci. USA* **100**, 13396–13401.

Milton, S. L., Manuel, L. and Lutz, P. L. (2003a). Slow death in the leopard frog *Rana pipiens*: neurotransmitters and anoxia tolerance. *J. Exp. Biol.* **206**, 4021–4028.

Minoli, S. A. and Lazari, C. R. (2003). Chronobiological basis of thermo-preference in the haematophagous bug *Triatoma infestans*. *J. Insect Physiol.* **49**, 927–932.

Misener, S. R., Chen, C.-P. and Walker, V. K. (2001). Cold tolerance and pro-line metabolic gene expression in *Drosophila melanogaster*. *J. Insect Physiol.* **47**, 393–400.

Moller, H. (1996). Lessons for invasion theory from social insects. *Biol. Conserv.* **78**, 125–142.

Mooney, H. A. and Hobbs, R. J. (2000). *Invasive Species in a Changing World*. Washington, DC: Island Press.

Moran, N. A. (1992). The evolutionary maintenance of alternative phenotypes. *Am. Nat.* **139**, 971–989.

Mori, I. (1999). Genetics of chemotaxis and thermotaxis in the nematode *Caenorhabditis elegans*. *Annu. Rev. Genet.* **33**, 399–422.

Morin, P., McMullen, D. C. and Storey, K. B. (2005). HIF-1$\alpha$ involvement in low temperature and anoxia survival by a freeze tolerant insect. *Mol. Cell. Biochem.* **280**, 99–106.

Murphy, B. F. (1986). The effect of acclimation temperature and the removal of peripheral temperature receptors on body temperature preference in the cock-roach, *Periplaneta americana*. *J. Thermal Biol.* **11**, 209–212.

Murphy, B. F. and Heath, J. E. (1983). Temperature sensitivity in the prothoracic ganglion of the cockroach *Periplaneta americana* and its relationship to thermo-regulation. *J. Exp. Biol.* **105**, 305–315.

Must, A., Merivee, E., Luik, A., Mand, M. and Heidemaa, M. (2006). Responses of antennal campaniform sensilla to rapid temperature changes in ground beetles of the tribe platynini with different habitat preferences and daily activity rhythms. *J. Insect Physiol.* **52**, 506–513.

Myers, J. H. (1988). Can a general hypothesis explain population cycles of forest Lepidoptera. *Adv. Ecol. Res.* **18**, 179–242.
Neargarder, G., Dahlhoff, E. P. and Rank, N. E. (2003). Variation in thermal tolerance is linked to phosphoglucose isomerase genotype in a montane leaf beetle. *Funct. Ecol.* **17**, 213–221.
Nedved, O. (1998). Modelling the relationship between cold injury and accumulated degree days in terrestrial arthropods. *Cryo Lett.* **19**, 267–274.
Ness, J. H. (2004). Forest edges and fire ants alter the seed shadow of an ant-dispersed plant. *Oecologia* **138**, 448–454.
Nielsen, M., Overgaard, J., Sorensen, J. G., Holmstrup, M., Justesen, J. and Loeschcke, V. (2005). Role of HSF activation for resistance to heat, cold and high-temperature knock-down. *J. Insect Physiol.* **51**, 1320–1329.
Nishikawa, M., Yokohari, F. and Ishibashi, T. (1992). Response characteristics of two types of cold receptors on the antennae of the cockroach, *Periplaneta americana*. *J. Comp. Physiol. A* **171**, 299–307.
Nishino, H., Yamashita, S., Yamazaki, Y., Nishikawa, M., Yokohari, F. and Mizunami, M. (2003). Projection neurons originating from thermo- and hygrosensory glomeruli in the antennal lobe of the cockroach. *J. Comp. Neurol.* **455**, 40–55.
North, R. D. (1991). Transpiration and humidity preference in a temperate wood ant *Formica rufa* L. *J. Insect Physiol.* **37**, 279–286.
Nylin, S. and Gotthard, K. (1998). Plasticity in life-history traits. *Annu. Rev. Entomol.* **43**, 63–83.
Oberhauser, K. and Peterson, A. T. (2003). Modeling current and future potential wintering distributions of eastern North American monarch butterflies. *Proc. Natl. Acad. Sci. USA* **100**, 14063–14068.
O'Dowd, D. J., Green, P. T. and Lake, P. S. (2003). Invasional 'meltdown' on an oceanic island. *Ecol. Lett.* **6**, 812–817.
Ottersen, G., Planque, B., Belgrano, A., Post, E., Reid, P. C. and Stenseth, N. C. (2001). Ecological effects of the North Atlantic oscillation. *Oecologia* **128**, 1–14.
Overgaard, J., Sørensen, J. G., Petersen, S. O., Loeschcke, V. and Holmstrup, M. (2005). Changes in membrane lipid composition following rapid cold hardening in *Drosophila melanogaster*. *J. Insect Physiol.* **51**, 1173–1182.
Owen-Smith, N. (2005). Incorporating fundamental laws of biology and physics into population ecology: the metaphysiological approach. *Oikos* **111**, 611–615.
Palmer, M., Bernhardt, E., Chornesky, E., Collins, S., Dobson, A., Duke, C., Gold, B., Jacobson, R., Kingsland, S., Kranz, R., Mappin, M., Martinez, M. L., Micheli, F., Morse, J., Pace, M., Pascual, M., Palumbi, S., Reichman, O. J., Simons, A., Townsend, A. and Turner, M. (2004). Ecology for a crowded planet. *Science* **304**, 1251–1252.
Park, T. (1962). Beetles, competition, and populations. *Science* **138**, 1369–1375.
Parkash, R., Tyagi, P. K., Sharma, I. and Rajpurohit, S. (2005). Adaptations to environmental stress in altitudinal populations of two *Drosophila* species. *Phys. Entomol.* **30**, 353–361.
Parker, J. C. (1993). In defense of cell volume? *Am. J. Physiol.* **265**, C1191–C1200.
Parmesan, C., Root, T. L. and Willig, M. R. (2000). Impacts of extreme weather and climate on terrestrial biota. *Bull. Am. Meteorol. Soc.* **81**, 443–450.
Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W. J., Thomas, J. A. and Warren, M. (1999). Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **399**, 579–583.
Parmesan, C. and Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37–42.

Parr, C. L., Sinclair, B. J., Andersen, A. N., Gaston, K. J. and Chown, S. L. (2005). Constraint and competition in assemblages: a cross continental and modeling approach for ants. *Am. Nat.* **165**, 481–493.

Parsell, D. A. and Lindquist, S. (1993). The function of heat-shock proteins in stress toleransb: degradation and reactivation of damaged proteins. *Annu. Rev. Genet* **27**, 437–496.

Parsons, P. A. (2005). Environments and evolution: interactions between stress, resource inadequacy and energetic efficiency. *Biol. Rev.* **80**, 589–610.

Patapouitian, A., Peier, A. M., Story, G. M. and Viswanath, V. (2003). ThermoTRP channels and beyond: mechanisms of temperature sensation. *Nat. Rev. Neurosci.* **4**, 529–539.

Patz, J. A., Graczyk, T. K., Geller, N. and Vittor, A. Y. (2000). Effects of environmental change on emerging parasitic diseases. *Int. J. Parasitol.* **30**, 1395–1405.

Pearson, R.G. and Dawson, T.P. (2003). Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecol. Biogeogr.* **12**, 361–371.

Perfecto, I. and Vandermeer, J. (1996). Microclimate changes and the indirect loss of ant diversity in a tropical agroecosystem. *Oecologia* **108**, 577–582.

Pétavy, G., David, J. R., Gibert, P. and Moreteau, B. (2001). Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. *J. Thermal Biol.* **26**, 29–39.

Piersma, T. and Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends Ecol. Evol.* **18**, 228–233.

Pigliucci, M. (2005). Evolution of phenotypic plasticity: where are we going now? *Trends Ecol. Evol.* **20**, 481–486.

Pigliucci, M. and Murren, C. J. (2003). Perspective: genetic assimilation and a possible evolutionary paradox: can macroevolution sometimes be so fast as to pass us by? *Evolution* **57**, 1455–1464.

Pigliucci, M., Murren, C. J. and Schlichting, C. D. (2006). Phenotypic plasticity and evolution by genetic assimilation. *J. Exp. Biol.* **209**, 2362–2367.

Pörtner, H. O. (2001). Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88**, 137–146.

Pörtner, H. O. (2002a). Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. *Comp. Biochem. Physiol. A* **132**, 739–761.

Pörtner, H. O. (2002b). Physiological basis of temperature-dependent biogeography: trade-offs in muscle design and performance in polar ectotherms. *J. Exp. Biol.* **205**, 2217–2230.

Pörtner, H. O., Hardewig, I., Sartoris, F. J. and van Dijk, P. L. M. (1998). Energetic aspects of cold adaptation: critical temperatures in metabolic, ionic and acid-base regulation? In: *Cold Ocean Physiology* (eds Pörtner, H. O. and Playle, R.), pp. 88–120. Cambridge: Cambridge University Press.

Pörtner, H. O., van Dijk, P. L. M., Hardewig, I. and Sommer, A. (2000). Levels of metabolic cold adaptation: tradeoffs in eurythermal and stenothermal ectotherms. In: *Antarctic Ecosystems: Models for Wider Ecological Understanding* (eds Davison, W., Howard-Williams, C. and Broady, P. A.), pp. 109–122. Christchurch, New Zealand: New Zealand Natural Sciences.

Pounds, J. A., Bustamante, M. R., Coloma, L. A., Consuegra, J. A., Fogden, M. P. L., Foster, P. N., La Marca, E., Masters, K. L., Merino-Viteri, A., Puschendorf, R., Ron, S. R., Sánchez-Azofeifa, G. A., Still, C. J. and Young, B. E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **439**, 161–167.

Prange, H. D. and Hamilton, G. B. (1992). Humidity selection by thermoregulating grasshoppers. *J. Therm. Biol.* **17**, 353–355.
Prosser, C. L. (1986). *Adaptational Biology: Molecules to Organisms*. New York: Wiley.
Pszczolkowski, M. A. and Chiang, A.-S. (2000). Effects of chilling stress on allatal growth and juvenile hormone synthesis in the cockroach, *Diploptera punctata*. *J. Insect Physiol.* **46**, 923–931.
Pszczolkowski, M. A. and Gelman, D. B. (2004). Chilling stress effects on corpus allatum proliferation in the Hawaiian cockroach, *Diploptera punctata*: a role for ecdysteroids. *J. Insect Physiol.* **50**, 203–208.
Pullin, A. S. (1996). Physiological relationships between insect diapause and cold toleransb: coevolution or coincidence? *Eur. J. Entomol.* **93**, 121–129.
Qin, S. F., Minami, Y., Hibi, M., Kurosaki, T. and Yamamura, H. (1997). Syk-dependent and – independent signaling cascades in B cells elicited by osmotic and oxidative stress. *J. Biol. Chem.* **272**, 2098–2103.
Qin, W., Tyshenko, M. G., Wu, B. S., Walker, V. S. and Robertson, R. M. (2003). Cloning and characterization of a member of the *hsp70* gene family from *Locusta migratoria* a highly thermotolerant insect. *Cell Stress Chaperon* **8**, 144–152.
Queitsch, C., Sangster, T. A. and Lindquist, S. (2002). Hsp90 as a capacitor of phenotypic variation. *Nature* **417**, 618–624.
Rako, L. and Hoffmann, A. A. (2006). Complexity of the cold acclimation response in *Drosophila melanogaster*. *J. Insect Physiol.* **52**, 94–104.
Ramirez, J. M., Elsen, F. P. and Robertson, R. M. (1999). Long-term effects of prior heat shock on neuronal potassium currents recorded in a novel insect ganglion slice preparation. *J. Neurophysiol.* **81**, 795–802.
Ramløv, H. (2000). Aspects of natural cold tolerance in ectothermic animals. *Hum. Reprod.* **15**, 26–46.
Randolph, S. E. and Rogers, D. J. (2000). Fragile transmission cycles of tick-borne encephalitis virus may be disrupted by predicted climate change. *Proc. R. Soc. Lond. B* **267**, 1741–1744.
Rees, C. J. C. (1970). The primary process of reception on the type 3 ('water') receptor cell of the fly, *Phormia terranovae*. *Proc. Roy. Soc. Lond. B* **174**, 469–490.
Relyea, R. A. (2002). Costs of phenotypic plasticity. *Am. Nat.* **159**, 272–282.
Renault, D., Nedved, O., Hervant, F. and Vernon, P. (2004). The importance of fluctuating thermal regimes for repairing chill injuries in the tropical beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) during exposure to low temperature. *Physiol. Entomol.* **29**, 139–145.
Richardson, D. M., Rouget, M., Ralston, S. J., Cowling, R. M., van rensburg, B. J. and Thuiller, W. (2005). Species richness of alien plants in South Africa: environmental correlates and the relationship with indigenous plant species richness. *Ecoscience* **12**, 391–402.
Rickman, J. K. and Connor, E. F. (2003). The effect of urbanization on the quality of remnant habitats for leaf-mining lepidoptera on *Quercus agrifolia*. *Ecography* **26**, 777–787.
Rinehart, J. P. and Denlinger, D. L. (2000). Heat-shock protein 90 is down-regulated during pupal diapause in the flesh fly, *Sarcophaga crassipalpis*, but remains responsive to thermal stress. *Ins. Mol. Biol.* **9**, 641–645.
Rinehart, J. P., Yocum, G. D. and Denlinger, D. L. (2000a). Thermotolerance and rapid cold hardening ameliorate the negative effects of brief exposures to high or low temperatures on fecundity in the flesh fly, *Sarcophaga crassipalpis*. *Physiol. Entomol.* **25**, 330–336.

Rinehart, J. P., Yocum, G. D. and Denlinger, D. L. (2000b). Developmental up-regulation of inducible hsp70 transcripts, but not the cognate form, during pupal diapause in the flesh fly, *Sarcophaga crassipalpis*. *Ins. Biochem. Mol. Biol.* **30**, 515–521.

Ring, R. A. and Danks, H. V. (1994). Desiccation and cryoprotection: overlapping adaptations. *Cryo Lett.* **15**, 181–190.

Roberts, C. S., Seely, M. K., Ward, D., Mitchell, D. and Campbell, J. D. (1991). Body temperatures of Namib Desert tenebrionid beetles: their relationship in laboratory and field. *Physiol. Entomol.* **16**, 463–475.

Robertson, M. P., Villet, M. H. and Palmer, A. R. (2004). A fuzzy classification technique for predicting species' distributions: applications using invasive alien plants and indigenous insects. *Divers. Distrib.* **10**, 461–474.

Robertson, R. M. (2004a). Modulation of neural circuit operation by prior environmental stress. *Integr. Comp. Biol.* **44**, 21–27.

Robertson, R. M. (2004b). Thermal stress and neural function: adaptive mechanisms in insect model systems. *J. Thermal Biol.* **29**, 351–358.

Robertson, R. M., Xu, H., Shoemaker, K. L. and Dawson-Scully, K. (1996). Exposure to heat shock affects thermosensitivity of the locust flight system. *J. Neurobiol.* **29**, 367–383.

Robinson, T., Rogers, D. and Williams, B. (1997a). Univariate analysis of tsetse habitat in the common fly belt of Southern Africa using climate and remotely sensed vegetation data. *Med. Vet. Entomol.* **11**, 223–234.

Robinson, T., Rogers, D. and Williams, B. (1997b). Mapping tsetse habitat suitability in the common fly belt of Southern Africa using multivariate analysis of climate and remotely sensed vegetation data. *Med. Vet. Entomol.* **11**, 235–245.

Roca, M. J. and Lazzari, C. R. (1994). Effects of relative humidity on the haematophagous bug *Triatoma infestans*: hygropreference and eclosion success. *J. Insect Physiol.* **40**, 901–907.

Roces, F. and Kleineidam, C. (2000). Humidity preference for fungus culturing by workers of the leaf-cutting ant *Atta sexdens rubropilosa*. *Insectes Soc.* **47**, 348–350.

Roces, F. and Nunez, J. A. (1995). Thermal sensitivity during brood care in workers of two *Camponotus* ant species: circadian variation and its ecological correlates. *J. Insect Physiol.* **41**, 659–669.

Roff, D. A. (1986). The evolution of wing dimorphism in insects. *Evolution* **40**, 1009–1020.

Roff, D. A. (2002). *Life History Evolution*. Sunderland, MA: Sinauer Associates.

Rogers, D. J. (2000). Satellites, space, time and the African trypanosomisases. *Adv. Parasitol.* **47**, 129–171.

Rogers, D. J. and Randolph, S. E. (1991). Mortality rates and population density of tsetse flies correlated with satellite imagery. *Nature* **351**, 739–741.

Rogers, D. J. and Randolph, S. E. (2000). The global spread of malaria in a future, warmer world. *Science* **289**, 1763–1766.

Rogers, D. J. and Randolph, S. E. (2002). A response to the aim of eradicating tsetse from Africa. *Trends Parasitol.* **18**, 534–536.

Rohmer, C., David, J. R., Moreteau, B. and Joly, D. (2004). Heat induced male sterility in *Drosophila melanogaster*: adaptive genetic variations among geographic populations and role of the Y chromosome. *J. Exp. Biol.* **207**, 2735–2743.

Rojas, R. R. and Leopold, R. A. (1996). Chilling injury in the house fly: evidence for the role of oxidative stress between pupariation and emergence. *Cryobiology* **33**, 447–458.

Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C. and Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. *Nature* **421**, 57–60.

Rosenzweig, M., Brennan, K. M., Tayler, T. D., Phelps, P. O., Patapoutian, A. and Garrity, P. A. (2005). The *Drosophila* ortholog of vertebrate TRPA1 regulates thermotaxis. *Genes Dev.* **19**, 419–425.

Roslin, T. and Kotze, D. J. (2005). Preface: insects and plants in space. *Ann. Zool. Fenn.* **42**, 291–294.

Rourke, B. C. (2000). Geographic and altitudinal variation in water balance and metabolic rate in a California grasshopper, *Melanoplus sanguinipes*. *J. Exp. Biol.* **203**, 2699–2712.

Roy, D. B., Rothery, P., Moss, D., Pollard, E. and Thomas, J. A. (2001). Butterfly numbers and weather: predicting historical trends in abundance and the future effects of climate change. *J. Anim. Ecol.* **70**, 201–217.

Rutherford, S. L. and Lindquist, S. (1998). Hsp90 as a capacitor for morphological evolution. *Nature* **396**, 336–342.

Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L. F., Jackson, R. B., Kinzig, A., Leemans, R., Lodge, D. M., Mooney, H. A., Oesterheld, M., Poff, N. L., Sykes, M. T., Walker, B. H., Walker, M. and Wall, D. H. (2000). Global biodiversity scenarios for the year 2100. *Science* **287**, 1770–1774.

Salin, C., Renault, D., Vannier, G. and Vernon, P. (2000). A sexually dimorphic response in supercooling temperature, enhanced by starvation, in the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). *J. Thermal Biol.* **25**, 411–418.

Salt, R. W. (1956). Influence of moisture content and temperature on cold-hardiness of hibernating insects. *Can. J. Zool.* **34**, 283–294.

Salt, R. W. (1966). Effect of cooling rate on the freezing temperatures of supercooled insects. *Can. J. Zool.* **44**, 655–659.

Salvucci, M. E. (2000). Sorbitol accumulation in whiteflies: evidence for a role in protecting proteins during heat stress. *J. Thermal Biol.* **25**, 353–361.

Salvucci, M. E., Stecher, D. S. and Henneberry, T. J. (2000). Heat shock proteins in whiteflies, an insect that accumulates sorbitol in response to heat stress. *J. Thermal Biol.* **25**, 363–371.

Samietz, J., Salser, M. A. and Dingle, H. (2005). Altitudinal variation in behavioural thermoregulation: local adaptation vs plasticity in California grasshoppers. *J. Evol. Biol.* **18**, 1087–1096.

Samways, M. J., Osborne, R., Hastings, H. and Hattingh, V. (1999). Global climate change and accuracy of prediction of species geographical ranges: establishment success of introduced ladybirds (Coccinellidae, *Chilocorus* spp.) worldwide. *J. Biogeogr.* **26**, 795–812.

Samways, M. J., Taylor, S. and Tarboton, W. (2005). Extinction reprieve following alien removal. *Conserv. Biol.* **19**, 1329–1330.

Sanders, N. J., Gotelli, N. J., Heller, N. E. and Gordon, D. M. (2003). Community disassembly by an invasive species. *Proc. Natl. Acad. Sci. USA* **100**, 2474–2477.

Sayeed, O. and Benzer, S. (1996). Behavioral genetics of thermosensation and hygrosensation in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **93**, 6079–6084.

Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Syst.* **24**, 35–68.

Schilman, P. E. and Lazzari, C. R. (2004). Temperature preference in *Rhodnius prolixus*, effects and possible consequences. *Acta Trop.* **90**, 115–122.

Schlichting, C. D. (2002). Phenotypic plasticity in plants. *Plant Species Biol.* **17**, 85–88.
Schlichting, C. D. and Pigliucci, M. (1998). *Phenotypic Evolution: A Reaction Norm Perspective*. Sunderland, MA: Sinauer Associates.
Schliess, F. and Haüssinger, D. (2002). The cellular hydration state: a critical determinant for cell death and survival. *Biol. Chem.* **383**, 577–583.
Schliess, F., Wiese, S. and Haüssinger, D. (1999). Osmotic regulation of the heat shock response in H4IIE rat hepatoma cells. *FASEB J.* **13**, 1557–1564.
Schneider, C. (2003). The influence of spatial scale on quantifying insect dispersal: an analysis of butterfly data. *Ecol. Entomol.* **28**, 252–256.
Seebacher, F. (2005). A review of thermoregulation and physiological performance in reptiles: what is the role of phenotypic flexibility? *J. Comp. Physiol. B* **175**, 453–461.
Segurado, P. and Araújo, M. B. (2004). An evaluation of methods for modelling species distributions. *J. Biogeogr.* **31**, 1555–1568.
Sejerkilde, M., Sørensen, J. G. and Loeschcke, V. (2003). Effects of cold- and heat hardening on thermal resistance in *Drosophila melanogaster*. *J. Insect Physiol.* **49**, 719–726.
Selås, V., Hogstad, O., Kobro, S. and Rafoss, T. (2004). Can sunspot activity and ultraviolet-B radiation explain cyclic outbreaks of forest moth pest species? *Proc. R. Soc. Lond. B* **271**, 1897–1901.
Sformo, T. and Doak, P. (2006). Thermal ecology of interior Alaska dragonflies (Odonata: Anisoptera). *Funct. Ecol.* **20**, 114–123.
Sgrò, C. M. and Partridge, L. (2000). Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *Am. Nat.* **156**, 341–353.
Shanbhag, S. R., Singh, K. and Singh, R. N. (1995). Fine structure and primary sensory projections of sensilla located in the sacculus of the antenna of *Drosophila melanogaster*. *Cell Tiss. Res.* **282**, 237–249.
Shear, W. A. and Kukalová-Peck, J. (1990). The ecology of Paleozoic terrestrial arthropods: the fossil evidence. *Can. J. Zool.* **68**, 1807–1834.
Shelford, V. E. (1911). Physiological animal geography. *J. Morphol.* **22**, 551–618.
Shields, V. D. C. (1994). Ultrastructure of the aporous sensilla on the galea of larval *Mamestra configurata* (Walker) (Lepidoptera: Noctuidae). *Can. J. Zool.* **72**, 2032–2054.
Shiga, S., Yasuyama, K., Okamura, N. and Yamaguchi, T. (2002). Neural- and endocrine control of flight muscle degeneration in the adult cricket, *Gryllus bimaculatus*. *J. Insect Physiol.* **48**, 15–24.
Shimada, K. and Riihimaa, A. (1990). Cold-induced freezing tolerance in diapausing and non-diapausing larvae of *Chymomyza costata* (Diptera: Drosophilidae) with accumulation of trehalose and proline. *Cryo Lett.* **11**, 243–250.
Shintani, Y. and Ishikawa, Y. (1999). Geographic variation in cold hardiness of eggs and neonate larvae of the yellow-spotted longicorn beetle *Psacothea hilaris*. *Physiol. Entomol.* **24**, 158–164.
Shreve, S. M., Kelty, J. D. and Lee, R. E. (2004). Preservation of reproductive behaviors during modest cooling: rapid cold-hardening fine-tunes organismal response. *J. Exp. Biol.* **207**, 1797–1802.
Sibly, R. M., Winokur, L. and Smith, R. H. (1997). Interpopulation variation in phenotypic plasticity in the speckled wood butterfly, *Pararge aegeria*. *Oikos* **78**, 323–330.
Simberloff, D. (2004). Community ecology: is it time to move on? *Am. Nat.* **163**, 787–799.

Simmons, A. D. and Thomas, C. D. (2004). Changes in dispersal during species' range expansions. *Am. Nat.* **164**, 378–395.
Sinclair, B. J. (1999). Insect cold tolerance: how many kinds of frozen? *Eur. J. Entomol.* **96**, 157–164.
Sinclair, B. J. (2001a). Field ecology of freeze tolerance: interannual variation in cooling rates, freeze–thaw and thermal stress in the microhabitat of the alpine cockroach *Celatoblatta quinquemaculata*. *Oikos* **93**, 286–293.
Sinclair, B. J. (2001b). Biologically relevant environmental data: macros to make the most of microclimate recordings. *Cryo Lett.* **22**, 125–134.
Sinclair, B. J., Addo-Bediako, A. and Chown, S. L. (2003a). Climatic variability and the evolution of insect freeze tolerance. *Biol. Rev.* **78**, 181–195.
Sinclair, B. J. and Chown, S. L. (2003). Rapid responses to high temperature and desiccation but not to low temperature in the freeze tolerant sub-Antarctic caterpillar *Pringleophaga marioni* (Lepidoptera, Tineidae). *J. Insect Physiol.* **49**, 45–52.
Sinclair, B. J. and Chown, S. L. (2005a). Climatic variability and hemispheric differences in insect cold tolerance: support from southern Africa. *Funct. Ecol.* **19**, 214–221.
Sinclair, B. J. and Chown, S. L. (2005b). Deleterious effects of repeated cold exposure in a freeze-tolerant sub-Antarctic caterpillar. *J. Exp. Biol.* **208** 879–969.
Sinclair, B. J., Klok, C. J. and Chown, S. L. (2004). Metabolism of the sub-Antarctic caterpillar *Pringleophaga marioni* during cooling, freezing and thawing. *J. Exp. Biol.* **207**, 1287–1294.
Sinclair, B. J. and Roberts, S. P. (2005). Acclimation, shock and hardening in the cold. *J. Thermal Biol.* **30**, 557–562.
Sinclair, B. J., Terblanche, J. S., Scott, M. B., Blatch, G. L., Klok, C. J. and Chown, S. L. (2006). Environmental physiology of three species of Collembola at Cape Hallett, North Victoria Land, Antarctica. *J. Insect Physiol.* **52**, 29–50.
Sinclair, B. J., Vernon, P., Klok, C. J. and Chown, S. L. (2003b). Insects at low temperatures: an ecological perspective. *Trends Ecol. Evol.* **18**, 257–262.
Slansky, F. and Rodriguez, J. G. (1987). *Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates*. New York: Wiley.
Somero, G. N. (1995). Proteins and temperature. *Annu. Rev. Physiol.* **57**, 43–68.
Somero, G. N. (2005). Linking biogeography to physiology: evolutionary and acclimatory adjustments of thermal limits. *Front. Zool.* **2**, 1–9.
Somero, G. N., Dahlhoff, E. and Lin, J. J. (1996). Stenotherms and eurytherms: mechanisms establishing thermal optima and tolerance ranges. In: *Animals and Temperature. Phenotypic and Evolutionary Adaptation* (eds Johnston, I. A. and Bennett, A. F.), pp. 53–78. Cambridge: Cambridge University Press.
Sømme, L. (1995). *Invertebrates in Hot and Cold Arid Environments*. Berlin: Springer.
Sømme, L. (1996). The effect of prolonged exposures at low temperatures in insects. *Cryoletters* **17**, 341–346.
Sømme, L. (1999). The physiology of cold hardiness in terrestrial arthropods. *Eur. J. Entomol.* **96**, 1–10.
Sørensen, J. G., Dahlgaard, J. and Loeschcke, V. (2001). Genetic variation in thermal tolerance among natural populations of *Drosophila buzzatii*: down regulation of Hsp70 expression and variation in heat stress resistance traits. *Funct. Ecol.* **15**, 289–296.
Sørensen, J. G., Michalak, P., Justesen, J. and Loeschcke, V. (1999). Expression of the heat-shock protein Hsp70 in *Drosophila buzzatii* lines selected for thermal resistance. *Hereditas* **131**, 155–164.

Sørensen, J. G., Norry, F. M., Scannapieco, A. C. and Loeschcke, V. (2005). Altitudinal variation for stress resistance traits and thermal adaptation in adult *Drosophila buzzatii* from the New World. *J. Evol. Biol.* **18**, 829–837.

Spicer, J. I. and Gaston, K. J. (1999). *Physiological Diversity and its Ecological Implications*. Oxford: Blackwell Science.

Stanley, S. M. (1989). *Earth and Life through Time*. 2nd edn New York: W.H. Freeman.

Steele, J. H. (1985). A comparison of terrestrial and marine ecological systems. *Nature* **313**, 355–358.

Steenkamp, H. E. and Chown, S. L. (1996). Influence of dense stands of an exotic tree, *Prosopis glandulosa* Benson, on a savanna dung beetle (Coleoptera: Scarabaeinae) assemblage in southern Africa. *Biol. Conserv.* **78**, 305–311.

Stefanescu, C., Herrando, S. and Paramo, F. (2004). Butterfly species richness in the north-west Mediterranean Basin: the role of natural and human-induced factors. *J. Biogeogr.* **31**, 905–915.

Steidle, J. L. M. and Reinhard, J. (2003). Low humidity as a cue for habitat preference in the parasitoid *Lariophagus distinguendus*. *BioControl* **48**, 169–175.

Stenseth, N. C. and Mysterud, A. (2005). Weather packages: finding the right scale and composition of climate in ecology. *J. Anim. Ecol.* **74**, 1195–1198.

Stenseth, N. C., Ottersen, G., Hurrell, J. W., Mysterud, A., Lima, M., Chan, K. S., Yoccoz, N. G. and Adlandsvik, B. (2003). Studying climate effects on ecology through the use of climate indices: the North Atlantic oscillation, El Niño southern oscillation and beyond. *Proc. R. Soc. Lond. B* **270**, 2087–2096.

Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science* **301**, 65.

Stillwell, R. C. and Fox, C. W. (2005). Complex patterns of phenotypic plasticity: interactive effects of temperature during rearing and oviposition. *Ecology* **86**, 924–934.

Stireman, J. O. III., Dyer, L. A., Janzen, D. H., Singer, M. S., Lill, J. T., Marquis, R. J., Ricklefs, R. E., Gentry, G. L., Hallwachs, W., Coley, P. D., Barone, J. A., Greeney, H. F., Connahs, H., Barbosa, P., Morais, H. C. and Diniz, I. R. (2005). Climatic unpredictability and parasitism of caterpillars: implications of global warming. *Proc. Natl. Acad. Sci. USA* **102**, 17384–17387.

Storch, D., Gaston, K. J. and Cepák, J. (2002). Pink landscapes: $1/f$ spectra of spatial environmental variability and bird community composition. *Proc. R. Soc. Lond. B* **269**, 1791–1796.

Storey, K. B. (1990). Biochemical adaptation for cold hardiness in insects. *Philos. Trans. R. Soc. Lond. B* **326**, 635–654.

Storey, K. B. (1996). Oxidative stress: animal adaptations in nature. *Brazil. J. Med. Biol. Res.* **29**, 1715–1733.

Storey, K. B. (1997). Organic solutes in freezing tolerance. *Comp. Biochem. Physiol. A* **117**, 319–326.

Storey, K. B. (2002). Life in the slow lane: molecular mechanisms of estivation. *Comp. Biochem. Physiol. A* **133**, 733–754.

Storey, K. B., Baust, J. G. and Storey, J. M. (1981). Intermediary metabolism during low temperature acclimation in the overwintering gall fly larva, *Eurosta solidaginis*. *J. Comp. Physiol.* **144**, 183–190.

Storey, J. M. and Storey, K. B. (1986). Winter survival of the gall fly larva, *Eurosta solidaginis*: profiles of fuel reserves and cryoportectants in a natural population. *J. Insect Physiol.* **32**, 549–556.

Storey, K. B. and Storey, J. M. (1996). Natural freezing survival in animals. *Annu. Rev. Ecol. Syst.* **27**, 365–386.

Storey, K. B. and Storey, J. M. (2004). Metabolic rate depression in animals: transcriptional and translational controls. *Biol. Rev.* **79**, 207–233.
Sultan, S. E. (2004). Promising directions in plant phenotypic plasticity. *Perspect. Plant Ecol. Evol. Syst.* **6**, 227–233.
Sultan, S. E. and Spencer, H. G.. (2002). Metapopulation structure favors plasticity over local adaptation. *Am. Nat.* **160**, 271–283.
Suzuki, Y. and Nijhout, H. F. (2006). Evolution of a polyphenism by genetic accommodation. *Science* **311**, 650–652.
Tachibana, S.-I., Numata, H. and Goto, S. G. (2005). Gene expression of heat-shock proteins (Hsp23, Hsp70 and Hsp90) during and after larval diapause in the blow fly *Lucilia sericata*. *J. Insect Physiol.* **51**, 641–647.
Tammariello, S. P., Rinehart, J. P. and Denlinger, D. L. (1999). Desiccation elicits heat shock protein transcription in the flesh fly, *Sarcophaga crassipalpis*, but does not enhance tolerance to high or low temperatures. *J. Insect Physiol.* **45**, 933–938.
Tatem, A. J., Bayliss, M., Mellor, P. S., Purse, B. V., Capela, R., Pena, I. and Rogers, D. J. (2003). Prediction of bluetongue vector distribution in Europe and North Africa using satellite imagery. *Vet. Microbiol.* **97**, 13–29.
Tauber, M. J., Tauber, C. A., Nyrop, J. P. and Villani, M. G. (1998). Moisture, a vital but neglected factor in the seasonal ecology of insects: hypotheses and tests of mechanisms. *Environ. Entomol.* **27**, 523–530.
Terblanche, J. S. and Chown, S. L. (2006). The relative contributions of developmental plasticity and adult acclimation to physiological variation in the tsetse fly, *Glossina pallidipes* (Diptera, Glossinidae). *J. Exp. Biol.* **209**, 1064–1073.
Terblanche, J. S., Klok, C. J., Krafsur, E. S. and Chown, S. L. (2006). Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera: Glossinidae): implications for distribution modeling. *Am. J. Trop. Med. Hyg.* **74**, 786–794.
Terblanche, J. S., Klok, C. J., Marais, E. and Chown, S. L. (2004). Metabolic rate in the whip-spider, *Damon annulatipes* (Arachnida: Amblypygi). *J. Insect Physiol.* **50**, 637–645.
Thanaphum, S. and Haymer, D. S. (1998). A member of the hsp70 gene family from the Mediterranean fruit fly, *Ceratitis capitata*. *Ins. Mol. Biol.* **7**, 63–72.
Thomas, C. D., Bodsworth, E. J., Wilson, R. J., Simmons, A. D., Davies, Z. G., Musche, M. and Conradt, L. (2001). Ecological and evolutionary processes at expanding range margins. *Nature* **411**, 577–581.
Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F. N., de Siqueira, M. F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A. S., Midgley, G. F., Miles, L., Ortega-Huerta, M. A., Peterson, A. T., Phillips, O. L. and Williams, S. E. (2004). Extinction risk from climate change. *Nature* **427**, 145–148.
Thomas, C. D., Singer, M. C. and Boughton, D. A. (1996). Catastrophic extinction of population sources in a butterfly metapopulation. *Am. Nat.* **148**, 957–975.
Tichy, H. (2003). Low rates of change enhance effect of humidity on the activity of insect hygroreceptors. *J. Comp. Physiol. A* **189**, 175–179.
Tichy, H. and Loftus, R. (1996). Hygroreceptors in insects and a spider: humidity transduction models. *Naturwissenschaften* **83**, 255–263.
Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W. H., Simberloff, D. and Swackhamer, D. (2001). Forecasting agriculturally driven global environmental change. *Science* **292**, 281–284.

Torrence, C. and Compo, G. P. (1998). A practical guide to wavelet analysis. *Bull. Am. Meteorol. Soc.* **79**, 61–78.

Tsutsui, N. D., Suarez, A. V., Holway, D. A. and Case, T. J. (2000). Reduced genetic variation and the success of an invasive species. *Proc. Natl. Acad. Sci. USA* **97**, 5948–5953.

Tsvetkova, N. M., Horváth, I., Török, Z., Wolkers, W. F., Balogi, Z., Shigapova, N., Crowe, L. M., Tablin, F., Vierling, E., Crowe, J. H. and Vigh, L. (2002). Small heat-shock proteins regulate membrane lipid polymorphism. *Proc. Natl. Acad. Sci. USA* **99**, 13504–13509.

Tufto, J. (2000). The evolution of plasticity and nonplastic spatial and temporal adaptations in the presence of imperfect environmental cues. *Am. Nat.* **156**, 121–130.

Van Kleunen, M. and Fischer, M. (2005). Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytol.* **166**, 49–60.

Vannier, G. (1994). The thermobiological limits of some freezing tolerant insects: the supercooling and thermostupor points. *Acta Oecol.* **15**, 31–42.

Van Tienderen, P. H. (1991). Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution* **45**, 1317–1331.

Van Tienderen, P. H. (1997). Generalists, specialists, and the evolution of phenotypic plasticity in sympatric populations of distinct species. *Evolution* **51**, 1372–1380.

Vasseur, D. A. and Yodzis, P. (2004). The color of environmental noise. *Ecology* **85**, 1146–1152.

Vernon, P. and Vannier, G. (2002). Evolution of freezing susceptibility and freezing tolerance in terrestrial arthropods. *Comptes Rendus Biol.* **325**, 1185–1190.

Viswanath, V., Story, G. M., Peier, A. M., Petrus, M. J., Lee, V. M., Hwang, S. W., Patapoutian, A. and Jegla, T. (2003). Opposite thermosensor in fruitfly and mouse. *Nature* **423**, 822–823.

Vittor, A. Y., Gilman, R. H., Tielsch, J., Glass, G., Shields, T., Lozano, W. S., Pinedo-Cancino, V. and Patz, J. A. (2006). The effect of deforestation on the human-biting rate of *Anopheles darlingi*, the primary vector of falciparum malaria in the Peruvian Amazon. *Am. J. Trop. Med. Hyg.* **74**, 3–11.

Voets, T., Droogmans, G., Wissenbach, U., Janssens, A., Flockerzi, V. and Nilius, B. (2004). The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. *Nature* **430**, 748–754.

Voituron, Y., Mouquet, N., de Mazancourt, C. and Clobert, J. (2002). To freeze or not to freeze? An evolutionary perspective on the cold-hardiness strategies of overwintering ectotherms. *Am. Nat.* **160**, 255–270.

Waldow, U. (1970). Electrophysiological investigations of moist, dry and cold receptors on antenna of migratory locust. *Z. Vergl. Physiol.* **69**, 249–283.

Walters, A. C. and Mackay, D. A. (2003). An experimental study of the relative humidity preference and survival of the Argentine ant, *Linepithema humile* (Hymenoptera, Formicidae): comparisons with a native *Iridomyrmex* species in South Australia. *Insectes Soc.* **50**, 355–360.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J.-M., Hoegh-Guldberg, O. and Bairlein, F. (2002). Ecological responses to recent climate change. *Nature* **416**, 389–395.

Wang, X.-H. and Kang, L. (2005). Differences in egg thermotolerance between tropical and temperate populations of the migratory locust *Locusta migratoria* (Orthoptera: Acridiidae). *J. Insect Physiol.* **51**, 1277.

Ward, D. and Seely, M. K. (1996). Behavioral thermoregulation of six Namib Desert tenebrionid beetle species (Coleoptera). *Ann. Entomol. Soc. Am.* **89**, 442–451.

Watanabe, M., Kikawada, T., Minagawa, N., Yukuhiro, F. and Okuda, T. (2002). Mechanism allowing an insect to survive complete dehydration and extreme temperatures. *J. Exp. Biol.* **205**, 2799–2802.

Watson, R. T. (ed.) (2002). *Climate Change 2001: Synthesis Report*. Cambridge: Cambridge University Press.

Webb, T. J., Gaston, K. J., Hannah, L. and Woodward, F. I. (2006). Coincident scales of forest feedback on climate and conservation in a diversity hot spot. *Proc. R. Soc. Lond. B* **273**, 757–765.

Weissling, T. J. and Giblindav, R. M. (1993). Water-loss dynamics and humidity preference of *Rhynchophorus cruentatus* (Coleoptera, Curculionidae) adults. *Environ. Entomol.* **22**, 93–98.

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* **20**, 249–278.

West-Eberhard, M. J. (2003). *Developmental Plasticity and Evolution*. New York: Oxford University Press.

West-Eberhard, M. J. (2005). Developmental plasticity and the origin of species differences. *Proc. Natl. Acad. Sci. USA* **102**, 6543–6549.

Westneat, M. W., Betz, O., Blob, R. W., Fezzaa, K., Cooper, W. J. and Lee, W.-K. (2003). Tracheal respiration in insects visualized with synchrotron X-ray imaging. *Science* **299**, 558–560.

Weston, P. A. and Hoffman, S. A. (1991). Humidity and tactile responses of *Sitophilus zeamais* (Coleoptera, Curculionidae). *Environ. Entomol.* **20**, 1433–1437.

Wiens, J. J. (2004). What is speciation and how should we study it? *Am. Nat.* **163**, 914–923.

Wiens, J. J. and Graham, C. H. (2005). Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu. Rev. Ecol. Evol. Syst.* **36**, 519–539.

Wikelski, M. and Cooke, S. J. (2006). Conservation physiology. *Trends Ecol. Evol.* **21**, 38–46.

Williams, J. B., Ruehl, N. C. and Lee, R. E. (2004). Partial link between the seasonal acquisition of cold-tolerance and desiccation resistance in the goldenrod gall fly *Eurosta solidaginis* (Diptera: Tephritidae). *J. Exp. Biol.* **207**, 4407–4414.

Williams, P., Hannah, L., Andelman, S., Midgley, G., Araújo, M., Hughes, G., Manne, L., Martinez-Meyer, E. and Pearson, R. (2005). Planning for climate change: identifying minimum-dispersal corridors for the Cape Proteaceae. *Conserv. Biol.* **19**, 1063–1074.

Willig, M. R., Kaufman, D. M. and Stevens, R. D. (2003). Latitudinal gradients of biodiversity: pattern, process, scale and synthesis. *Annu. Rev. Ecol. Syst.* **34**, 273–309.

Willmer, P. G. (1982). Microclimate and the environmental physiology of insects. *Adv. Ins. Physiol.* **16**, 1–57.

Willmer, P., Stone, G. and Johnston, I. A. (1999). *Environmental Physiology of Animals*. Oxford: Blackwell Science.

Wilson, R. S. and Franklin, C. E. (2002). Testing the beneficial acclimation hypothesis. *Trends Ecol. Evol.* **17**, 66–70.

Wolfe, G. R., Hendrix, D. L. and Salvucci, M. E. (1998). A thermoprotective role for sorbitol in the silverleaf whitefly, *Bemisia argentifolii*. *J. Insect Physiol.* **44**, 597–603.

Wolfrum, U. (1997). Cytoskeletal elements in insect sensilla. *Int. J. Ins. Morphol. Embryol.* **26**, 191–203.

Woods, H. A. (1999). Patterns and mechanisms of growth of fifth-instar *Manduca sexta* caterpillars following exposure to low- or high-protein food during early instars. *Physiol. Biochem. Zool.* **72**, 445–454.

Woods, H. A. and Harrison, J. F. (2001). The beneficial acclimation hypothesis versus acclimation of specific traits: physiological change in water stressed *Manduca sexta*. *Physiol. Biochem. Zool.* **74**, 32–44.
Woods, H. A. and Harrison, J. F. (2002). Interpreting rejections of the beneficial acclimation hypothesis: when is physiological plasticity adaptive? *Evolution* **56**, 1863–1866.
Woods, H. A. and Hill, R. I. (2004). Temperature-dependent oxygen limitation in insect's eggs. *J. Exp. Biol.* **207**, 2267–2276.
Woods, H. A., Makino, W., Cotner, J. B., Hobbie, S. E., Harrison, J. F., Acharya, K. and Elser, J. J. (2003). Temperature and the chemical composition of poikilothermic organisms. *Funct. Ecol.* **17**, 237–245.
Worland, M. R. (1996). The relationship between water content and cold tolerance in the arctic collembolan *Onychiurus arcticus* (Collembola: Onychiuridae). *Eur. J. Entomol.* **93**, 341–348.
Worland, M. R. (2005). Factors that influence the freezing in the sub-Antarctic springtail *Tullbergia antarctica*. *J. Insect Physiol.* **51**, 881–894.
Worland, M. R. and Block, W. (2003). Desiccation stress at sub-zero temperatures in polar terrestrial arthropods. *J. Insect Physiol.* **49**, 193–203.
Worland, M. R., Grubor-Lajsic, G. and Montiel, P. (1998). Partial desiccation induced by sub-zero temperatures as a component of the survival strategy of the Arctic collembolan *Onychiurus arcticus* (Tullberg). *J. Insect Physiol.* **44**, 211–219.
Worland, M. R., Leinaas, H.-P. and Chown, S. L. (2006). Supercooling point frequency distributions in Collembola are affected by moulting. *Funct. Ecol.* **20**, 323–329.
Worland, M. R., Wharton, D. A. and Byars, S. G. (2004). Intracellular freezing and survival in the freeze tolerant alpine cockroach *Celatoblatta quinquemaculata*. *J. Insect Physiol.* **50**, 225–232.
Wu, B. S., Lee, J. K., Thompson, K. M., Walker, V. K., Moyes, C. D. and Robertson, R. M. (2002). Anoxia induces thermotolerance in the locust flight system. *J. Exp. Biol.* **205**, 815–827.
Xenopoulos, M. A., Lodge, D. M., Alcamo, J., Märker, M. L., Schulze, K. and van Vuuren, D. (2005). Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Global Change Biol.* **11**, 1557–1564.
Xu, H. and Robertson, R. M. (1994). Effects of temperature on properties of flight neurons in locust. *J. Comp. Physiol. A* **175**, 193–202.
Yamamoto, A. H. (1994). Temperature preference of *Drosophila immigrans* and *D. virilis*: intra- and inter-population genetic variation. *Jpn. J. Gen.* **69**, 67–76.
Ybarrondo, B. A. (1995). Habitat selection and thermal preference in two species of water scavenger beetles (Coleoptera: Hydrophilidae). *Physiol. Zool.* **68**, 749–771.
Yi, S.-X. and Lee, R. E. (2003). Detecting freeze injury and seasonal cold-hardening of cells and tissues in gall fly larvae, *Eurosta solidaginis* (Diptera, Tephritidae) using fluorescent vital dyes. *J. Insect Physiol.* **49**, 999–1004.
Yi, S.-X. and Lee, R. E. (2004). *In vivo* and *in vitro* rapid cold-hardening protects cells from cold-shock injury in the flesh fly. *J. Comp. Physiol. B* **174**, 611–615.
Yocum, G. D. (2001). Differential expression of two HSP70 transcripts in response to cold shock, thermoperiod, and adult diapause in the Colorado potato beetle. *J. Insect Physiol.* **47**, 1139–1145.
Yocum, G. D. and Denlinger, D. L. (1992). Prolonged thermotolerance in the flesh fly, *Sarcophaga crassipalpis*, does not require continuous expression or persistence of the 72 kDa heat-shock protein. *J. Insect Physiol.* **38**, 603–609.

Yocum, G. D., Joplin, K. H. and Denlinger, D. L. (1991). Expression of heat shock proteins in response to high and low temperatures in diapausing pharate larvae of the gypsy moth, *Lymantria dispar*. *Archiv. Insect Biochem. Physiol.* **18**, 239–249.

Yocum, G. D., Joplin, K. H. and Denlinger, D. L. (1998). Upregulation of a 23 kDa small heat shock protein transcript during pupal diapause in the flesh fly *Sarcophaga crassipalpis*. *Ins. Biochem. Mol. Biol.* **28**, 677–682.

Yocum, G. D., Kemp, W. P., Bosch, J. and Knoblett, J. N. (2005). Temporal variation in overwintering gene expression and respiration in the solitary bee *Megachile rotundata*. *J. Insect Physiol.* **51**, 621–629.

Yocum, G. D., Ždárek, J., Joplin, K. H., Lee, R. E., Smith, D. C., Manter, K. D. and Denlinger, D. L. (1994). Alteration of the eclosion rhythm and eclosion behavior in the flesh fly, *Sarcophaga crassipalpis*, by low and high temperature stress. *J. Insect Physiol.* **40**, 13–21.

Yoder, J. A., Benoit, J. B., Denlinger, D. L. and Rivers, D. B. (2006). Stress-induced accumulation of glycerol in the flesh fly, *Sarcophaga bullata*: evidence indicating anti-desiccant and cryoprotectant functions of this polyol and a role for the brain in coordinating the response. *J. Insect Physiol.* **52**, 202.

Yokahari, F. (1978). Hygroreceptor mechanism in the antenna of the cockroach *Periplaneta*. *J. Comp. Physiol.* **124**, 53–60.

Zachariassen, K. E. (1985). Physiology of cold tolerance in insects. *Physiol. Rev.* **65**, 799–832.

Zamudio, K. R., Huey, R. B. and Crill, W. D. (1995). Bigger isn't always better: body size, developmental and parental temperature and male territorial success in *Drosophila melanogaster*. *Anim. Behav.* **49**, 671–677.

Zatsepina, O. G., Velikodvorskaia, V. V., Molodtsov, V. B., Garbuz, D., Lerman, D. N., Bettencourt, B. R., Feder, M. E. and Evgenev, M. B. (2001). A *Drosophila melanogaster* strain from sub-equatorial Africa has exceptional thermotolerance but decreased Hsp70 expression. *J. Exp. Biol.* **204**, 1869–1881.

Zavaleta, E. S., Hobbs, R. J. and Mooney, H. A. (2001). Viewing invasive species removal in a whole-ecosystem context. *Trends Ecol. Evol.* **16**, 454–459.

Zeilstra, I. and Fischer, K. (2005). Cold tolerance in relation to developmental and adult temperature in a butterfly. *Physiol. Entomol.* **30**, 92–95.

Zera, A. J. and Denno, R. F. (1997). Physiology and ecology of dispersal polymorphism in insects. *Annu. Rev. Entomol.* **42**, 207–231.

Zera, A. J. and Zhao, Z. W. (2003). Morph-dependent fatty acid oxidation in a wing-polymorphic cricket: implications for the trade-off between dispersal and reproduction. *J. Insect Physiol.* **49**, 933–943.

Zhao, Z. and Zera, A. J. (2002). Differential lipid biosynthesis underlies a tradeoff between reproduction and flight capability in a wing-polymorphic cricket. *Proc. Natl. Acad. Sci. USA* **99**, 16829–16834.

Zhao, Z. and Zera, A. J. (2004a). The hemolymph JH titer exhibits a large-amplitude, morph-dependent, diurnal cycle in the wing-polymorphic cricket, *Gryllus firmus*. *J. Insect Physiol.* **50**, 93–102.

Zhao, Z. and Zera, A. J. (2004b). A morph-specific daily cycle in the rate of JH biosynthesis underlies a morph-specific daily cycle in the hemolymph JH titer in a wing-polymorphic cricket. *J. Insect Physiol.* **50**, 965–973.

Ziegler, A. and Altner, H. (1995). Are the most numerous sensilla of terrestrial isopods hygroreceptors? Ultrastructure of the dorsal tricorn sensilla of *Porcellio scaber*. *Cell Tiss. Res.* **282**, 135–145.

# Nest Thermoregulation in Social Insects

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## Abstract

Most social insect species are able to regulate the temperature within their nests. In this review, we examine the variety of mechanisms that social insect species have evolved to regulate temperature. We divide these mechanisms into two broad categories: active and passive. 'Passive' temperature regulation includes such mechanisms as nest site selection to optimize internal nest temperature, nest structures that permit passive heating or cooling, or simple behaviour such as brood translocation to regions within a nest where temperatures are most favourable. 'Active' temperature regulation refers to behaviour where individuals modify nest temperature by physical activity like wing fanning or evaporative cooling.

Although there is enormous variation in the thermoregulatory mechanisms, there are also many similarities. All thermoregulatory mechanisms are self-organized and arise from simple rules followed by each individual worker.

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## 1 Introduction

Part of the ecological success of social insects (all termites and ants, and some wasps and bees) is that they have at least some ability to regulate temperatures within their nests (Wilson, 1971). This often allows them to be physically active even when non-social insects of similar size would either be moribund with cold or seeking refuge from heat. Many social insect species regulate their nest temperatures within specific, sometimes very narrow, boundaries, despite extremes in ambient temperature. Honey bee colonies, for example, are able to maintain brood nest temperatures within the range of 33–36 °C, even when the ambient temperature ranges from well below freezing to above 45 °C (e.g. Himmer, 1932; Lindauer, 1955; Fahrenholz *et al.*, 1989).

If nest temperatures are not kept within the species-specific boundaries, there are often undesirable consequences. In some termites and ants, for example, the growth of the fungi that they cultivate for food may be affected (Powell and Stradling, 1986; Korb and Linsenmair, 2000a; Bollazzi and Roces, 2002). In many species, abnormalities can develop in the brood, or adults may not emerge at all (Himmer, 1927, 1932; Brian, 1963, 1973; Jay, 1963; Ishay, 1973; Kronenberg and Heller, 1982; Roces and Nunez, 1989; Tautz *et al.*, 2003; Jones *et al.*, 2005; McMullan and Brown, 2005). In honey bees, even slightly atypical brood rearing temperatures can affect the behaviour of the bees as adults (Tautz *et al.*, 2003; Jones *et al.*, 2005).

In this review we examine the wide variety of mechanisms that social insect species have evolved to regulate the temperature of their nests and brood (Table 1). We divide these mechanisms into two broad categories: active and passive. 'Passive' temperature regulation includes such mechanisms as nest site selection to optimize internal nest temperature, nest structures that permit passive heating or cooling, or simple behaviour such as brood translocation to regions within a nest where temperatures are most favourable. 'Active' temperature regulation refers to behaviour where individuals modify nest temperature by physical activity like wing fanning (Fig. 1) or evaporative cooling.

Both active and passive thermoregulation requires mechanisms that coordinate the activities of individual workers so that the outcome is a stable or more stable brood nest temperature. For active mechanisms of thermoregulation, this is obvious: the number of workers engaged in cooling or heating behaviour must rise and fall according to the current temperature within the nest. Less obviously, the same holds true for passive mechanisms. For example, a termite mound is constructed by tens of thousands of workers, none of which has any perception of the overall shape of the mound, or its intricately engineered ventilation tunnels and appropriate solar orientation. Thus, systems of passive nest thermoregulation

TABLE 1 Active and passive thermoregulatory systems used in different social insect groups (see text for details)

| Social Insect Group | Passive Thermoregulation | | |
|---|---|---|---|
| | Nest orientation | Nest architecture | Nest site selection/ colony emigration |
| Ants | S | S | M |
| Bees: Honey bees | S | S | A |
| Stingless bees | ? | M | A |
| Bumble bees | S | A | A |
| Wasps: Polistinae | ? | S | A |
| Vespinae | ? | A? | A |
| Termites | S | S | S |

| Social Insect Group | Active Thermoregulation | | | |
|---|---|---|---|---|
| | Clustering/ generating metabolic heat | Direct incubation | Fanning | Water evaporation |
| Ants | S | N | N | N |
| Bees: Honey bees | A | A* | A | A |
| Stingless bees | S? | ? | S | N |
| Bumble bees | A | A | A | N |
| Wasps: Polistinae | ? | N? | A | A |
| Vespinae | M | M | A | S |
| Termites | S | N | N | N |

S = Some species
M = Most species
A = All species
N = No species
*This behaviour has not been examined in all species, but we suggest that most, if not all, honey bee species incubate their brood directly.

require the coordination of the activities of workers that build the nest, so that the colony-level outcome of stable brood nest temperatures is achieved.

The mechanisms that coordinate the activities of individual workers are best understood from the principles of self-organization, which describe how a system can acquire order and structure "through interactions internal to the system without intervention by external directing influences" (Camazine *et al.*, 2001). Below we will review our current understanding of how the behaviour of individuals acting independently can nonetheless result in the emergent property of a stable nest temperature, and the apparent importance of inter-individual variance in generating stability. We begin, however, with a survey of the mechanisms of nest thermoregulation, both passive and active, that have evolved across social insect taxa.

FIG. 1 A honey bee (*Apis mellifera*) worker fanning at the entrance of her nest (Photo by M. Ricketts).

## 2 Passive mechanisms

Nest site selection, nest orientation and nest architecture are the primary mechanisms used by social insects to regulate their nest's microclimate. These passive mechanisms provide a buffer between brood nest temperature and ambient temperature, and therefore greatly affect the ease with which workers can regulate the temperature inside their nest by more energetically expensive active mechanisms. For example, Korb and Linsenmair (2000a) found that the coefficients of variation were not significantly different in internal nest temperature of the African termite species *Macrotermes bellicosus,* is not different between occupied and unoccupied mounds (occupied: 0.54 °C, unoccupied: 0.48 °C). However, mean mound temperatures are lower (~27 °C) in nests heated only by the sun compared with nests with active termites and fungi (~30 °C). In this section, we provide examples of the variety of passive mechanisms used by social insects for nest thermoregulation.

### 2.1 NEST SITE SELECTION AND COLONY EMIGRATION

Nest site selection plays a major role in the ability of social insect colonies to maintain stable nest temperatures. Nest site choice falls into two broad categories. For many species, the main criterion when selecting a nest site is physical protection against environmental perturbations; others select sites where the microclimate provides a relatively stable temperature.

### 2.1.1 *Bees*

Some honey-bee and all bumble-bee and stingless-bee species nest in cavities; predominantly tree hollows, but also in disused rodent burrows, cavities in old termite nests and under the leaf litter. These cavities provide insulation, thereby helping colonies to retain metabolic heat and providing protection from variations in ambient temperature (Wille and Michener, 1973; Heinrich, 1979; Engels *et al.*, 1995). However, the open-nesting honey bees, which occur in tropical environments, build a nest in the open, choosing sites with appropriate levels of solar radiation. As the open-nesting honey bee species form the basal clades of the honey bee tribe (Engel and Schultz, 1997; Arias and Sheppard, 2005), the colonization of temperate areas may have been facilitated by the move into protective cavities (Ruttner, 1988; Oldroyd and Wongsiri, 2006).

In cavity-nesting western honey bees (*A. mellifera*), cavity selection by swarms is based on a variety of attributes. In general, favoured cavities have a volume of at least 15 l, an entrance with a sunny aspect, a small entrance size (smaller than 75 $cm^2$), an entrance on the floor of the cavity and an elevation of several metres ( > 3 m) above the ground (Seeley, 1976, 1977; Avitabile *et al.*, 1978; Seeley and Morse, 1978; Jaycox and Parise, 1980, 1981; Rinderer *et al.*, 1981, 1982; Schmidt and Hurley, 1995; Camazine *et al.*, 1999). All of these properties enhance nest thermoregulation. Inside the cavity, the workers build a number of vertical combs out of wax, the upper and peripheral areas of which are used for storing pollen and honey while the centre contains the brood (Seeley, 1976; Camazine, 1991). The central location of the brood area means that the brood is insulated by the surrounding honey store.

The giant mountain honey bee (*A. laboriosa*) prefers nest sites with a southerly aspect to build its single exposed comb (Underwood, 1986, 1990). Similarly Doedikar *et al.* (1977) and Reddy (1993) found a strong tendency for established nests of the common giant honey bee *Apis dorsata* to be orientated in a north-south direction (Fig. 2). Presumably, this maximizes solar radiation and minimizes exposure to cold winds (Oldroyd and Wongsiri, 2006). The dwarf honey bees *A. florea* and *A. andreniformis* select shaded sites (Wongsiri *et al.*, 1997) and will migrate their nest if it subsequently becomes exposed to the sun (Seeley *et al.*, 1982).

For many stingless bee species, selecting an appropriate cavity appears to be the primary mechanism for keeping nest temperatures optimal, despite high ambient temperatures. For example, in southern Africa, all *Trigona* species nest inside a cavity, either deep underground (60–100 cm in *T. denoiti*) or inside a tree hollow. The nest depth of *T. denoiti* and insulating properties of the tree cavity in the case of *T. griboidoi* imply that the nests are rarely thermally stressed (Moritz and Crewe, 1988).

FIG. 2 The giant honey bee *Apis dorsata* selects sheltered nest sites like this one on an apartment building in Bangalore India (Photo by B. Oldroyd).

### 2.1.2 *Wasps*

Social wasps nest in a variety of locations, including underground and tree cavities, and in enclosed and open nests suspended from tree branches (Ishay, 1973). Microclimate has been found to influence nest site selection and reproductive strategies in *Polistes* wasps. Females of the temperate species *Polistes fuscatus* are more likely to initiate nests in warm sites. Such sites lead to earlier production of workers and larger founding groups, than cool sites (Jeanne and Morgan, 1992). These authors suggest that larger founding groups occur because warm sites are more attractive to female joiners and usurpers. In addition, philopatry may have evolved due to selection acting on founding females to nest in climatically favourable sites.

### 2.1.3 *Ants*

Ants are one of the most diverse insect suborders comprising 70% of all social insect species. This diversity is reflected in the range of environments they occupy, from deep in soil to forest canopies (Holldobler and Wilson, 1990). We identify three main strategies by which ant species regulate nest temperatures. First, like many social bees, some ant species rely on protection from a cavity, such as a tree stump or underground burrow (Chen *et al.*, 2002). Second, some species migrate their nests frequently, varying the amount of cover they select, depending on the temperature and season (Ofer, 1970; Kuriachan and Vinson, 2000; Miyata *et al.*, 2003). Third, still others move their brood to areas of optimal temperature within the same nest (Roces and Nunez, 1989, 1995; Bollazzi and Roces, 2002; Pranschke and Hooper-Bui, 2003).

In some ant species, nest location is important for regulating nest temperature and also affects additional forms of temperature control. In

northern Idaho, the carpenter ant *Camponotus vicinus*, for example, nests mostly in fallen logs and tree stumps (Chen *et al*., 2002). These ants select open dry sites, where the temperature is significantly higher, over closed canopy areas (Chen *et al*., 2002). Similarly, for the wood ant *Formica polyctena*, a temperate species of northern Europe and Scandinavia, nest site choice is an important aspect of nest temperature regulation and also affects the nest heating strategy used by the ants. At dry sites, the ants utilize solar radiation to heat their nests. In addition to orientating the mound to maximize incident solar radiation, the workers maximize this effect by basking on the outside of the mound. The dry nest material has a low thermal capacity and so the thermal energy in the workers' bodies can increase the nest temperature, especially in the evening when many heated workers return to the interior of the nest (Frouz, 2000). The surface of the dry nest also provides insulation as there is a low loss of thermal energy during the night. In contrast, nests at wet and shaded sites utilize the decomposing plant matter in their mounds as a source of heat (Frouz, 2000).

Nest migration is perhaps the most common method of temperature control used by ants. Migratory species adjust the amount of cover they select for their nest, depending on the temperature and season. The primitive ponerine ant *Onychomyrmex hedleyi*, for example, occurring in the highland rainforests of North Queensland, Australia, migrates frequently (Miyata *et al*., 2003). Similarly, the weaver ant *Polyrhachis simplex* of Israel, migrates seasonally, in the winter and early spring (Ofer, 1970), and colonies of the polygynous form of the red imported fire ant (*Solenopsis invicta*) often migrate their nests in response to changing ambient temperatures (Kuriachan and Vinson, 2000). Both *O. hedleyi* and *P. simplex* increase the amount of protection selected for their nests in the cooler seasons. *O. hedleyi* colonies bivouac in the upper leaf litter layer in the warm and rainy seasons, from November to March. In the cool and dry season, from April to October, most colonies bivouac in the lower litter layer or up to 12 cm below the ground in natural cavities and cracks or holes dug by other insects or earthworms (Miyata *et al*., 2003). During the summer, *P. simplex* ants build their nests in the litter of dried leaves, under stones or in the hollow of a tree stump. In winter, the majority of nests are located within cavities (Ofer, 1970). The transient nature of the nests, in both these species, implies that selecting appropriate cover is a critical thermoregulatory mechanism.

Although an appropriate brood incubation temperature is primarily achieved by selecting an appropriate site for the nest, brood incubation temperature is precisely controlled by workers moving the brood from one part of that nest to another, depending on the brood's stage of development and temperatures in different parts of the nest (Roces and Nunez, 1989, 1995; Bollazzi and Roces, 2002; Pranschke and Hooper-Bui, 2003) (Fig. 3). This occurs both in species with large, permanent nests or mounds, and in species with more transient nests.


FIG. 3 A *Solenopsis invicta* mound, opened on the side closest to the sun, showing the placement of brood during mid-morning (Photo courtesy of W. Tschinkel. Reprinted by permission of the publisher from THE FIRE ANTS by Walter S. Tschinkel, Plate 10, Cambridge Mass.: The Belknap Press of Harvard University Press, Copyright © 2006 by the President and Fellows of Harvard College). (A) Intact mound, (B) sunny-side with the mound surface removed, (C) mound cut in vertical section, after surface removal. During mid-morning, the temperature just under the mound surface was ~30 °C while in the mound core it was 23 °C (Tschinkel, 2006).

*Camponotus mus* nests under stones or in rotting wood on the ground, where colonies experience large temperature fluctuations. Rather than constantly moving the brood around within the nest as the temperature changes, *C. mus* nurse workers regulate the temperature of their brood by moving it to the preferred temperature at two fixed times – the hottest and coolest times of the day (Roces and Nunez, 1995). The brood is therefore kept well within the daily maximum and minimum, but experience considerable fluctuations in temperature throughout the day. Using artificial nests housed in a 12 h/12 h light/dark cycle, with two temperature options (27.5 °C and 30.8 °C) known to be selected by the ants in relation to the light:dark cycle, Roces and Nunez (1989) found that a total of four to five workers translocated the brood twice a day. One translocation occurred in the middle of the light period (at 30.8 °C), when the highest environmental temperatures occur, and again 8 h later, during the night (at 27.5 °C) (Roces and Nunez, 1989, 1995). Thus, the threshold of thermal tolerance in *C. mus* workers is related to the time of day. Highest thermal sensitivity occurs during the two daily translocation times: the middle of the photophase and two hours after the onset of the dark period. At these times, workers are more likely to transport brood in response to minor temperature changes. For example, shortly after 2 p.m., nurses began moving the brood as soon as the temperature was increased experimentally by 0.1–0.2 °C above the selected temperature of 30.8 °C; whereas, later in the day (anticipating a fall in temperature?), nurse workers will tolerate temperatures up to 3.7 °C, above 30.8 °C. Nurse workers of *C. rufipes*, a dominant species in subtropical and tropical regions of South America where temperature fluctuations are less marked, also show some circadian rhythmicity in sensitivity to temperature changes, but retain a constant preference. Nurse workers have a higher tolerance for high temperatures in the middle

of the light period, when differences of 6.7–7.8 °C above the mean preferred temperature (25 °C) are tolerated (Roces and Nunez, 1995). In the dark period, on the other hand, workers are more sensitive to temperature increases and begin to move the brood as soon as the temperature averages 1.9 °C above 25 °C. However, *C. rufipes* show no rhythm in their translocation of brood along an artificial thermal gradient in their nest (Roces and Nunez, 1995).

Similar brood and fungal garden translocations, in response to temperature variation, are carried out by workers in species with more permanent nests (Weber, 1957; Navarro and Jaffe, 1985; Lapointe *et al*., 1998; Roces and Kleineidam, 2000; Bollazzi and Roces, 2002; Pranschke and Hooper-Bui, 2003). Workers of the thatching grass cutting ant *Acromyrmex heyeri*, for example, construct a thatch mound with dry grass and soil that protects a central fungus garden. The thatch mound provides insulation, but as with *C. mus* and *C. rufipes*, workers also translocate the brood and fungal food that they culture, in response to low and high temperatures (Bollazzi and Roces, 2002). Similarly, in red imported fire ant (*S. invicta*) populations of Louisiana, the presence of brood in the above-ground section of the nest (the mound) is strongly associated with mound temperature (Pranschke and Hooper-Bui, 2003) (Fig. 3). *S. invicta* brood are only found in mounds when mound temperatures are between 25 °C and 30 °C. The brood is moved by workers to subterranean parts of the nest when the temperature in the above-ground section exceeds 32 °C (Pranschke and Hooper-Bui, 2003). By using a controlled temperature gradient, Bollazzi and Roces (2002) showed that *A. heyeri* uses a similar strategy: workers quickly translocate all items of brood and fungus when the temperature exceeds 36 °C, and prefer temperatures between 24 °C and 25 °C. For *A. heyeri,* the probability of brood and fungus removal was less at the lower end of the temperature gradient (10 °C) and the temperatures selected by the workers were also slightly lower, 22 °C and 21 °C. Brief exposure to low temperatures may not compromise the growth and development of the fungus (Bollazzi and Roces, 2002).

Migration within the nest is also used by some termites to track the best brood rearing temperatures (Cabrera and Rust, 1996; Cabrera and Kamble, 2001).

### 2.2 NEST ORIENTATION

In some termite and ant species, nest temperatures are moderated by nest orientation. Nest orientation often influences the amount of solar radiation absorbed by a nest and the time of day that the highest radiation is received. Many species orientate their nests so that it is warmed by solar radiation in the cool of the morning. Other species orientate the nest so that it offers the smallest possible profile to incident solar radiation during the middle of the day (Table 1).

### 2.2.1 *Termites*

Two termite species (*Amitermes meridionalis* and *A. laurensis*) occurring in northern Australia rely on the structure and orientation of their mounds for regulating nest temperature. These termites build wedge-shaped 'magnetic' mounds where the long axis of the mounds is oriented north–south (Grigg and Underwood, 1977; Jacklyn, 1992) (Fig. 4). Mean mound orientation differs significantly between populations, depending on their longitude. Jacklyn (1992) changed the orientation of some mounds by giving them 'a nudge with a four-wheel drive vehicle'. He showed that the temperature gradient between the east and west faces was significantly altered by the change in orientation. Similarly, the rate of cooling on the eastern face during the afternoon was consistently affected by mound orientation. During the cooler dry season, mounds of natural orientation experience rapid morning heating on their eastern face, followed by a temperature plateau until the sun sets. Thus, the geographic variation in mean mound orientation is an adaptive response to environmental variation across northern Australia (Jacklyn, 1992). During the dry season, when daily minimum temperatures decline, large numbers of termites, including workers, larvae and reproductive nymphs, move to the eastern face in the morning and stay there during the day, probably to reduce the variation in temperature they experience.

### 2.2.2 *Ants*

The orientation and shape of ant nests influence the amount of solar radiation that reaches the surface of the nests (Hubbard and Cunningham, 1977). Imported fire ant (*S. invicta*) mounds are oval in shape, with the majority of the long axes oriented north–south. This shape and orientation means that the sides of the mound with the greatest surface area face the sun early in the morning and late in the afternoon. Nest mounds of *Formica ulkei* near Chicago are also asymmetrically shaped, with the long slope aligned so that it receives maximum solar radiation (Scherba, 1958). If a nest is experimentally shaded to alter the aspect receiving greatest radiation intensity, the ants adjust the alignment of the longest slope of the nest. A further example is found in the nests of *Formica truncorum* in northern Norway, where workers only place nesting material against the southern face of tree stumps (Elton, 1932).

Mounds of the North American harvester ant *Pogonomyrmex occidentalis* are constructed to maximize exposure to solar radiation on one slope of the dome and minimize it on another (Cole, 1994). The nest cones slope more towards the south and east because the peak of the mound is displaced from centre to the north and west. The mainly south–eastern direction of the nest cone increases the collection of solar radiation in the


FIG. 4 Magnetic termite, *Amitermes meridionalis*, mounds in Litchfield National Park, North West Australia. The long axix is oriented (A) north-south, and (B) east-facing parts are heated by the morning sun. (C) Several of these mounds can be found in the same area (Photos courtesy of Nathan Lo).

morning (Cole, 1994). In addition, the nest entrance is oriented towards the south–east, allowing workers at the nest entrance to make maximum use of solar radiation falling on the south–eastern slope. Interestingly, these adaptations result in a range of temperatures (although not as extreme as ambient temperature variation) within the mound. The temperature of the nest 5 cm below the ground varies from 12.9 °C to 41.8 °C while the range

of temperature fluctuation at a depth of 5 cm below the peak of the cone varies between 10.7 °C and 43.3 °C. Workers appear to take advantage of the range of temperatures available by moving their brood to appropriate areas of the nest cone (Cole, 1994), similar to the brood movements of the ant species discussed above.

### 2.3 NEST ARCHITECTURE

Social insects' nests vary enormously in both structure and materials, but the different designs all have a common end point – maintenance of a more stable brood nest temperature than ambient. Some structural features are important for retaining heat, while others are effective in dissipating it.

#### 2.3.1 *Bees*

In many bee species, the nest is insulated by the cavity chosen as the nest site. More precise temperature regulation is achieved through the construction of protective layers around the nest, comb shape and brood position in the comb.

Bumble bees, add materials such as grass and plant parts to the nest in order to improve insulation. In addition, the workers build a canopy of wax over the nest to trap metabolic heat. If the temperature in the nest increases, the workers may partially remove the wax canopy.

Similarly, many stingless bee species build nests insulated by three main layers (Fig. 5). First, the cavity is lined with batumen (a Portugese word meaning "wall") made of propolis (plant gums, saps or resins collected outside the hive) or a mixture of wax and propolis, and sometimes vegetable matter and mud (Wille and Michener, 1973). The batumen seals the nest cavity, except for the entrance and, in some species, ventilating holes. The batumen layer, is also used to seal off sections of the cavity that are too large for the nest (Wille and Michener, 1973). Second, immediately within the batumen lining there is often a layer of storage pots for pollen and honey made of cerumen (a mixture of wax and propolis). Third, inside the layer of storage pots there is an involucrum made of thin vertical leaves of cerumen joined to each other and to the pots. Usually, the involucrum encloses the brood comb completely (Wille and Michener, 1973).

The involucrum section of the nest is very important for heat conservation in many stingless bee species (Fletcher and Crewe, 1981; Roubik and Peralta, 1983; Engels *et al.*, 1995). *Melipona* build more involucrum in cooler climates than in equatorial forests (Engels *et al.*, 1995; Roubik, 2006). In *T. denoiti* nests, the layers of the involucrum act as baffles, which inhibit air movement and reduce loss of heat by convection (Fletcher and Crewe, 1981). Similarly, in *S. postica* the leaves of the involucrum provide

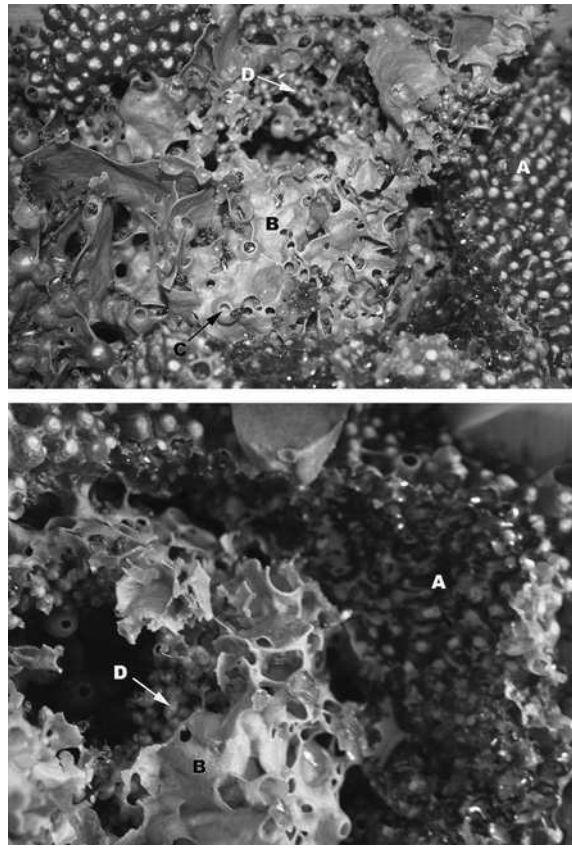


FIG. 5 Nest structure of *Austroplebeia australis*. The main nest layers for this species are the storage pots and involucrum enclosing the brood cells. (A) Honey pots, (B) involucrum, (C) storage pots (honey or pollen), and (D) brood cells (Photos courtesy of Peter Oxley).

effective insulation – during cool nights the temperature difference between the outer and inner layers of the involucrum (a distance of 1 cm) can be as much as 5 °C (Engels *et al.*, 1995). Also, in *Melipona rufiventris* and *M. seminigra* nests, in Brazil, the temperature inside the involucrum near the brood fluctuates less than ambient (ranging from 31 °C to 32.3 °C, where ambient ranged from ~23 °C to 30 °C) (Roubik and Peralta, 1983). The ability of *Melipona* species to maintain stable nest temperatures is mostly achieved by the involucrum, which traps much of the heat produced by the metabolism of the brood (Roubik and Peralta, 1983). Other stingless bee species, such as *Leurotrigona muelleri* and *Frieseomelitta varia*, do not build closely packed brood combs, but construct brood cells in a loosely-joined matrix. It seems likely that a spiral brood comb efficiently conserves heat

generated by the brood, whereas the matrix-style of brood cell construction facilitates heat dissipation (Fletcher and Crewe, 1981; Engels *et al*., 1995). Interestingly, three sibling species of Australian stingless bees, temperate to tropical *Trigona carbonaria*, sub-tropical *T. davenporti* and tropical *T. hockingsi* (Franck *et al*., 2004) differ strikingly in the construction of their brood comb, with *T. hockingsi* and *T. davenporti* constructing an open matrix of brood cells, whereas temperate *T. carbonaria* builds a densely packed spiral brood comb (Fig. 6). It remains to be seen if these two hugely variant forms of nest construction arise from environmental (temperature) cues or if these are truly species-specific traits.

In those stingless bee species that construct nests in the open, the nest is covered by insulating layers of batumen. Open-nesting species such as *Trigona corvina* and *T. spinipes* may also construct openings in the batumen, which are probably used for ventilation and temperature regulation (Wille and Michener, 1973).


FIG. 6 Nest structure of two stingless bee species (*Trigona hockingsi* and *T. carbonaria*). (A) *T. hockingsi* builds an open matrix of brood comb and (B) *T. carbonaria* builds densely packed spiral brood comb (Photos by B. Oldroyd).

Other aspects of nest architecture also help regulate the temperature in stingless bee nests. Species that construct a compact spiral brood comb (Fig. 6) utilize small cavities for nesting and can thus reduce the amount of heat lost via convection relative to species that utilize larger cavities. In addition, the absence of wax pillars used for access to the combs allows for a reduction in the space between the combs (Fletcher and Crewe, 1981). In many species, the entrance tube, which projects from the substrate or the nest surface in exposed nests, is closed over at night with soft cerumen from around the entrance. Closing the nest is mostly useful for defence but may also aid temperature regulation (Wille and Michener, 1973; Chinh *et al.*, 2005; Roubik, 2006). Species occurring in subtropical regions sometimes leave the opening closed for several days in cold weather (Wille and Michener, 1973). The long narrow entrance tube in species such as *T. denoiti* encumbers cooling by fanning, but the depth of the nests (60–100 cm) in the ground is sufficient to achieve a stable nest temperature even when ambient temperatures are high (Moritz and Crewe, 1988). When Moritz and Crewe (1988) recorded the temperature in nests of *T. denoiti* in Transvaal, South Africa, at an outside air temperature of 60.9 °C the internal temperature of a *T. denoiti* nest remained at 32 °C (Moritz and Crewe, 1988).

### 2.3.2 *Wasps*

Some social wasp species also build specific structures, sometimes within existing cavities, which help maintain stable nest temperatures. These mechanisms vary from constructing combs inside a cavity to building an exposed comb of heat-conserving shape and size, and even producing heat-generating thermoelectric pupal caps.

Species in the wasp subfamilies Vespinae and Polistinae utilize different thermoregulatory mechanisms and differ in their abilities to regulate nest temperature. These differences are mainly due to the contrasting nest architecture between the two groups. Polistinae nests consist of exposed comb, so effective thermoregulation of the brood relies predominantly on the environment at the site chosen for the nest (Jeanne and Morgan, 1992). Vespinae nests are enclosed by a thick paper jacket, which for many species is constructed inside a naturally-occurring cavity. Therefore, heat generated inside the nest can be stored and the nest temperature regulated (Gibo *et al.*, 1974a, 1974b; Seeley and Heinrich, 1981; Martin, 1988, 1992).

Foundresses or *Polistes riparius* of northern Japan, build what has been called a 'functional envelope' (Yamane and Kawamichi, 1975). The foundress constructs a number of empty cells at the side and lower periphery of the vertical nests and elongates the cells beyond the length of the pupal cocoons (Fig. 7). Other species, also occurring in cooler regions, construct their nests in this way (Yamane, 1988; Hozumi and Yamane, 2001). Hozumi and Yamne (2001) used paper models under field conditions to

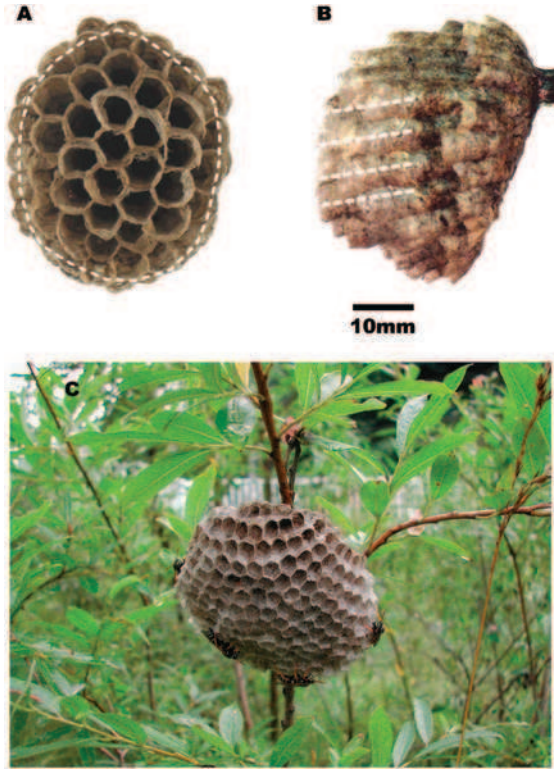


FIG. 7 The 'functional envelope' of the nest comb of *Polistes riparius* (Photos courtesy of Satoshi Hozumi). (A) View from the cell entrance of a *P. riparius* nest, the white circle indicates the brood area of the nest bordered by empty cells. (B) Lateral view of *P. riparius* nest, white lines show elongation of the cells beyond cocoon lengths. The nest shown in (A) and (B) was collected during the founding stage, just before the emergence of workers. (C) A mature nest of *P. riparius* in the field.

identify the role of the functional envelope in thermoregulation. Models with more or longer cells maintained temperatures above ambient for most of the night (Hozumi and Yamane, 2001). The extra cells presumably increase the ability of the colony to retain warmth, and provide some protection from wind and dew.

Comb shape also influences the thermal characteristics of exposed wasp nests. Yamane (1988) compared brood cell temperatures between two nest architectures in the tropical region of Padang, Sumatera Barat – the slender vertical comb of *Ropolidia variegata jacobsoni* and the oblong vertical comb of *R. fasciata*. When exposed to solar radiation, the slender combs of *R. variegata* were significantly cooler than those of *R. fasciata*. This difference probably arises because at least one side of the wall of all

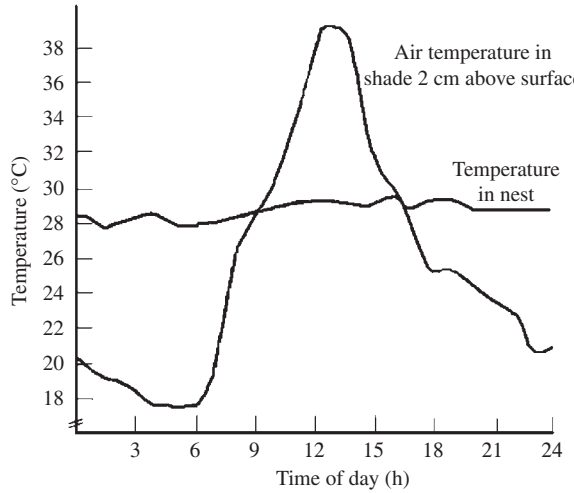


FIG. 8 The effect of exposure of the nest of the hornet *Vespa orientalis* to solar radiation during a 24 h period (courtesy Professor Jacob S. Ishay, reproduced with permission of Elsevier (Ishay and Barenholz-Paniry, 1995) and Rentokil Pest Control). The silk pupal caps help keep the temperature of the pupa stable.

cells of *R. variegata* are exposed, which facilitates radiation of heat (Yamane, 1988). This difference may explain the preferred nest sites of these two species. *R. variegata* often nests at sites constantly exposed to direct sunlight, whereas *R. fasciata* nests in shady sites, like the underside of broad evergreen leaves (Yamane, 1988).

The silk caps of pupal cells of *Vespa orientalis* assist in thermoregulating the brood (Ishay and Barenholz-Paniry, 1995). There are two main characteristics of the silk coating and the layer between the silk and pupa that help in thermoregulation. First, the silk layer insulates the pupae. Second, it acts as an energy accumulator which stores electrical charge during periods when there is heat available and releases the energy as heat during cooler temperatures (Ishay and Barenholz-Paniry, 1995). Figure 8 shows that the silk surrounding the pupae, particularly the silk caps, help regulate pupal temperature. The regulating effect of the silk is also localized and cells containing no brood (and having no cap) have a lower temperature (Joseph and Ishay, 2004).

### 2.3.3 *Termites*

Termite nests are often significant structures, which include intricate features for controlling the temperature of the chambers within. In mound-building species, variation in wall thickness, mound surface design or projecting structures, and as we have already seen, mound orientation,

are some of the nest characteristics that help provide a stable nest temperature. In addition, termite mound architecture is important for gas exchange.

The nest architecture of the termite *Macrotermes bellicosus* is an important contributor to effective nest thermoregulation. This species inhabits the Comoé National Park of the north-eastern Ivory Coast in West Africa, where there are two main habitat types: shrub savannah and gallery forest (Korb and Linsenmair, 1998b). Temperatures in the shrub savannah are generally higher and more variable than in the gallery forest. *M. bellicosus* appropriately adjusts the architecture of its nest to optimize nest thermoregulation in these different habitats. Mounds in the warmer savannah are relatively thin-walled and 'decorated' with numerous ridges and turrets. By contrast, mounds in open stands of the cooler gallery forest are dome-shaped and have thick walls with few projecting structures (Fig. 9). Thus, relative to the heat-dissipating architecture of the savannah mounds, mounds in the forest have reduced surface area and retain more heat (Korb and Linsenmair, 1998a,b, 1999).

Korb and Linsenmair (1998b) demonstrated the importance of nest architecture in thermoregulation by reducing the level of shading of forest-dwelling mounds, so that they experienced temperatures more like those in the savannah. In response to this manipulation, workers increased the surface complexity of their mounds until they resembled mounds in the savannah. Mounds in the gallery forest were found to have higher heat capacities than mounds of similar height in the shrub savannah, due to their thick walls. The temperature at the centre of the nest, where the brood chambers, fungus garden and the royal cell are, are kept at about 30 °C in the shrub savannah habitat. The cooler forest habitat, and ventilation requirements (see below), means that the interior of the mounds of the gallery forest are about 2 °C lower than those in the shrub savannah, despite the structural modifications to the nest's exterior. This means that the gallery forest is a suboptimal thermal habitat because 30 °C is the optimal temperature for the growth and development of termites, and for fungus cultivation (Korb and Linsenmair, 1998b).

The architecture of termite mounds is also important for effective ventilation of the nest and a large variety of nest structures are used to control ventilation; from enormous chimney projections, to air passages close to the surface in enclosed mounds with no chimney (Luscher, 1961; Weir, 1973; Darlington, 1984; Turner, 1994, 2001; Korb and Linsenmair, 2000b). The structure of the nest must satisfy the dual needs of effective ventilation and temperature regulation. For example, Korb and Linsenmair (2000b) showed that there is a trade-off between temperature regulation and gas exchange in the mounds of *M. bellicosus* in the cooler habitat of the gallery forest. In the forest habitat, the termites construct dome-shaped mounds with thick walls and reduced surface areas to reduce heat loss, but the

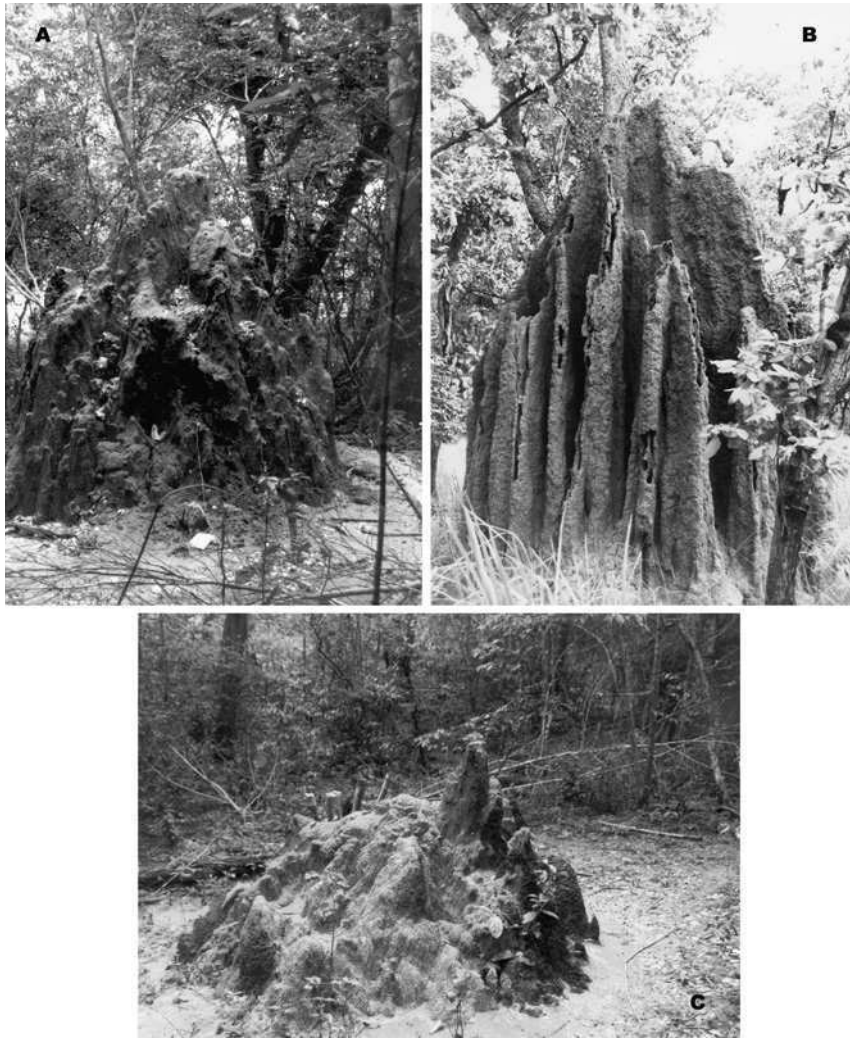


FIG. 9 *Macrotermes bellicosus* mounds in the Comoé National Park, West Africa (Photos courtesy of Judith Korb, reproduced with permission of Springer Science and Business Media; Korb, 2003). (A) Large gallery forest mound, (B) large savannah mound, and (C) small gallery forest mound.

reduction in surface area is constrained by the need to exchange respiratory gases; termites and their fungi have a high metabolic rate. In these mounds, gas exchange is limited almost entirely to the surface at the crest of the mound. By contrast, in savannah mounds, temperature does not restrict the amount of surface area available for gas exchange and respiratory gases are exchanged by holes across the entire surface of the mound.

Consequently, the interior of forest mounds have higher $CO_2$ concentrations than savannah mounds (Korb and Linsenmair, 2000b).

## 3 Active nest thermoregulation

In addition to passive thermoregulatory mechanisms, at least some species from most major social insect taxa actively heat or cool their nests. Some behaviour, such as clustering and generating metabolic heat to keep the colony warm at cool ambient temperatures (ants, termites, bees and wasps) and fanning of the wings to drive warm air out of the colony at warm ambient temperatures (bees and wasps), are common to different species, and even orders.

### 3.1 ACTIVE COLONY RESPONSES TO LOW TEMPERATURES

#### 3.1.1 *Bees*

One of the behavioural responses of most, if not all, social bee species to low temperatures is clustering. Workers are able to maintain stable brood nest temperatures at low ambient temperatures by forming tight clusters on or around the brood area and generating metabolic heat. Workers adjust the cluster shape and density by moving closer together or further apart, allowing them to fine-tune their response to temperature change.

Clustered bees generate metabolic heat (Free and Simpson, 1963; Fahrenholz *et al*., 1989), primarily by rapidly contracting and releasing their thoracic flight muscles (Kronenberg and Heller, 1982), while the muscles are disengaged from the wings. Below an ambient temperature of about 15 °C, honey bee (*A. mellifera*) workers, for example, gather together in a compact spherical cluster covering the brood (Kronenberg and Heller, 1982). Clustering reduces colony heat loss because the surface area available for heat exchange is minimized (Seeley, 1985). Both cavity- and open-nesting Asian honey bees have similar clustering behaviour to the European honey bee (Dyer and Seeley, 1991). For example, in the open-nesting species *A. florea* and *A. dorsata*, the workers who form a curtain over the comb move closer together at low ambient temperatures (Fig. 10). In *A. mellifera*, the threshold for clustering may be lower in the absence of brood than when it is present (Kronenberg and Heller, 1982). During winter, when there is no brood, variations in the colony temperature is larger than when the brood is present, despite the fact that workers still form clusters and generate metabolic heat using their flight muscles (Fahrenholz *et al*., 1989; Stabentheiner *et al*., 2003).

Stingless bees use strategies similar to that of honey bees for warming their nests. When exposed to cool ambient temperatures *Trigona denoiti* and

FIG. 10 *Apis florea* colony (Photo courtesy of Nadine Chapman). Workers that form the protective curtain covering the comb move closer together at low ambient temperatures, and further apart at high ambient temperatures.

*Scaptotrigona postica* workers are able to generate heat within the nest, presumably by clustering on the brood and quivering their flight muscles (Fletcher and Crewe, 1981; Engels *et al.*, 1995). However, *S. postica* also uses other mechanisms to retain thermal energy; workers gather small pieces

of cerumen in their mandibles and plaster the coldest area of the brood nest with a thick layer. Also, foraging is curtailed and the comb surface is covered with 1–2 layers of slowly moving bees (Engels *et al.*, 1995).

### 3.1.2 *Wasps*

Like honey-bee colonies, mature colonies of Vespine wasps can maintain constant nest temperatures (Gibo *et al.*, 1974a,b; Makino and Yamane, 1980; Martin, 1988, 1992). As their nests are enclosed, heat generated inside can be retained to keep the nest warm. Adult wasps congregate on top of the brood to increase nest temperature (Ishay, 1973) and probably produce heat in a similar fashion to honey-bee workers. In addition, adults also warm the nest by blowing warm air from the tracheal openings towards the pupae in their cocoons or even on pupae that have been extracted from their cocoons (Ishay and Barenholz-Paniry, 1995). The larvae of hornet brood are also thought to play a role in nest warming by activating their muscles (Ishay and Barenholz-Paniry, 1995). However, in some species, the ability of a colony to produce heat and maintain an elevated nest temperature depends on the season and reproductive stage of the colony. After the production of reproductives, and when the nest population declines, the thermoregulatory ability of the colony may also decline (Gibo *et al.*, 1974a,b). Martin (1988) suggests that the key to maintaining a constant nest temperature ($\sim$29 °C in *Vespa simillima xanthoptera*) is the maintenance of the colony biomass/worker activity above a level where heat production is greater than heat loss, even in the absence of the founding queen.

### 3.1.3 *Termites*

Some termites generate metabolic heat and cluster together to achieve stable nest temperatures. In the Australian termites *Coptotermes acinaciformis* and *C. frenchi*, the bark and wood of the living tree, and the walls of the nursery provide effective insulation, retaining heat within the nursery (Greaves, 1964). In colonies of both species, a difference of up to 20 °C is recorded between the metabolically-generated temperature at the centre of the nursery and the centre of an uncolonized tree (Greaves, 1964). The area of elevated temperature in the nursery is smaller in winter than in summer, suggesting that the termites aggregate in winter (Greaves, 1964).

### 3.1.4 *Ants*

Ants also gather together and generate heat. Similar to bees, both open- and mound-nesting species use comparable mechanisms for maintaining stable nest temperatures.

On cold days in early spring, workers of the red wood ant *Formica* species often engage in 'sunning behaviour' in which clusters of workers aggregate on the nest surface to absorb solar radiation (see above). However, in a behaviour remarkably similar to that of clustering honey bees and Vespine wasps, *Formica* workers in large nests are able to aggregate at the centre of their nest where they can generate an internal nest temperature of 25–30 °C, even when the ambient temperature is around 0 °C (Rosengren *et al.*, 1987). Thus, large nests are mostly independent of the need for solar radiation to achieve nest temperatures at which workers can be metabolically active. In contrast, workers in smaller colonies must engage in basking to allow nests to achieve a temperature where workers can be active. In large colonies, the heat-dependent activation of ant metabolism after the winter dormancy period may rely on positive feedback, where once some of the ants become warm enough for their metabolism to be activated, their activity warms other workers, and this can take place within the nest rather than relying on basking (Rosengren *et al.*, 1987).

Army ants form nests or bivouacs in which the bodies of workers make up the shelter by interlocking their tarsal claws. The thermoregulatory properties of bivouacs have been studied in *Eciton hamatum* and *E. burchelli*, two neotropical species that nest above the ground in open cavities, such as under the trunks of fallen trees (Schneirla *et al.*, 1954; Jackson, 1957; Franks, 1989). The workers of both species maintain a uniform temperature in the centre of the bivouac where the brood is located. For example, *E. burchelli* workers maintain the central nest temperature at about 28 °C with a variation of only 2 °C, although the ambient temperature of their rainforest habitat only varies by about 6–7 °C (peaking at 27 °C and falling to a minimum of 22 °C). In *E. burchelli* colonies, the heat required to maintain optimal temperatures for the brood is attained by the metabolic activity of the workers within the bivouac. Workers regulate the temperature by forming or closing ventilation channels within the nest structure (Franks, 1989). Bivouacs also change shape diurnally, becoming smaller and reducing their surface area to volume ratio when it is cold (Franks, 1989). Thermoregulation in honey-bee swarms (see Section 4.1) has remarkable parallels with army ant bivouacs.

## 3.2 DIRECT INCUBATION

### 3.2.1 *Bees and wasps*

In embryo nests of *Vespa simillima*, queens warm their brood by 2.5–4 °C above ambient by 'curling' behaviour, in which the queen curls her body around the pedicel of the nest (Makino and Yamane, 1980). When the first larvae appear, they too contribute to heat production in the colony (Gibo

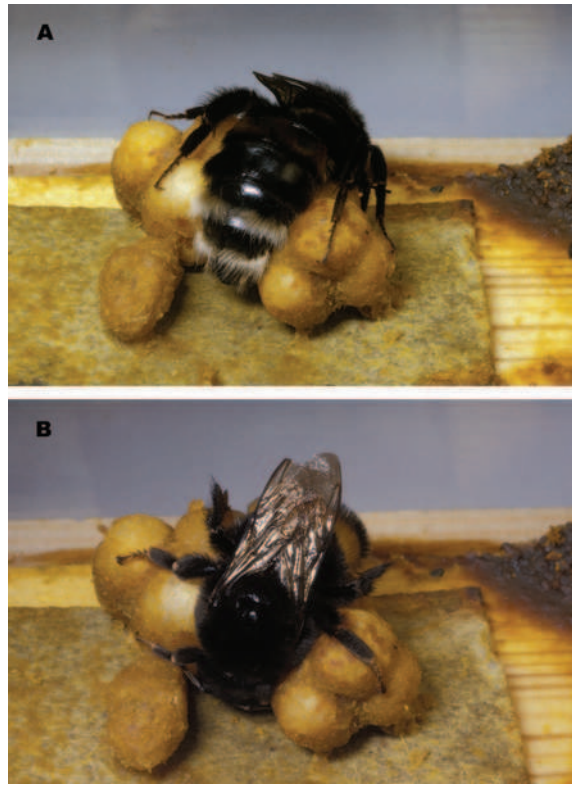


FIG. 11 A bumble bee (*Bombus terrestris*) queen incubating her brood clump (A) and (B) (Photos courtesy of Madeleine Beekman).

*et al*., 1977). Hornet workers directly incubate the brood by entering empty cells adjacent to pupae and placing their abdomen against the cocoon and pumping their abdomen to generate heat (Ishay, 1973).

During colony founding in bumble bees, the queen wraps herself around the brood clump and faces towards the honey pot whenever she is not foraging (Fig. 11). The queen presses her abdomen on to the brood clump to both insulate and incubate the brood (Heinrich, 1974a).While incubating, the queen produces heat in her thorax and distributes it to her abdomen (Heinrich and Kammer, 1973) by abdominal contractions (Heinrich, 1979). She also deposits a pheromone when she lays eggs, which allows her to restrict her incubation efforts to the area of the nest that contains the brood (Heinrich, 1974b). As the colony size increases, adult bees (both workers and drones) incubate the nymphs. By modulating metabolic activity, adults are able to regulate their abdominal temperature and therefore maintain the brood temperature within a narrow range (Heinrich, 1972). Incubating bees maintain their thorax (which provides

heat flow to the abdomen) between 34.5 °C and 37.5 °C even when the ambient temperature varies from 3 °C to 33 °C.

Incubation behaviour in response to low temperatures has also been reported for individual honey-bee workers (Bujok *et al*., 2002). Incubating workers assume a crouched posture, in which they press their warm thoraces onto the brood cell caps. Bujok *et al*. (2002) used thermographic infrared images of brood comb to reveal 'hot spots' on the capped brood area, where individual workers had been pressing their thoraces. Similar to social wasps, workers also heat the brood by entering empty cells directly adjacent to brood cells and maintaining a warm thoracic temperature (Kleinhenz *et al*., 2003).

## 3.3 ACTIVE COLONY RESPONSES TO HIGH TEMPERATURES

### 3.3.1 *Bees and wasps*

Social insects, predominantly bees and wasps, also use behavioural responses to cool moderately high nest temperatures (Table 1). The most common cooling behaviours, possible only in species where workers are alate, are wing fanning (where workers fan their wings, while standing in a stationary position, to drive warm air away from the nest, as shown in Fig. 1) and evaporative cooling (where workers collect water and place droplets on the surface of the brood comb).

At high ambient temperatures, both cavity- and open-nesting honey-bee workers fan the nest with their wings (Hazelhoff, 1954; Dyer and Seeley, 1991). Cavity-nesting bees fan on the combs inside their nest and also at the nest entrance, while open-nesting bees fan on the surface of workers that form the protective curtain over the nest (Oldroyd *et al*., 1994). Fanning direction appears to be important in temperature regulation. For example, *Apis mellifera* workers face towards the nest when fanning, whereas *A. cerana* and *A. koschevnikovi*, which are also cavity-nesting species, workers face away from the nest (Sakagami, 1960). In the open-nesting dwarf species *A. florea*, workers fan facing up the comb, whereas *A. andreniformis*, another open-nesting dwarf species, workers fan facing down the comb (Thapa and Wongsiri, 1994). It would be interesting to model what effect fanning direction has on nest thermoregulation.

Bumble bees, stingless bees, and *Polistes* and *Vespula* wasps also fan their nests to regulate nest temperature (Hasselrot, 1960; Moritz and Crewe, 1988; Jeanne and Morgan, 1992; Hunt *et al*., 1995; Riabinin *et al*., 2004; Roubik, 2006).

Honey bees, paper wasps and hornets use water evaporation to cool their nests. In this behaviour, workers distribute water throughout the nest, placing it in the small hollows that form on the margins of capped brood cells (Lindauer, 1954; Ishay and Barenholz-Paniry, 1995). Honey bees

FIG. 12 An *Apis dorsata* colony. Workers are spread out on the comb to reduce brood nest temperature (Photo by Ben Oldroyd).

(both cavity and open nesting) also spread water across the rim of open cells. Tongue lashing is also employed, in which workers draw a water droplet into a thin layer with their tongue (Lindauer, 1954; Dyer and Seeley, 1991; Jacklyn, 1992). Effective evaporative cooling requires appropriate coordination between water foragers and workers using water in the nest (Lindauer, 1954; Moritz and Southwick, 1992). The unloading time experienced by returning water foragers provides them with information on the water demands of the colony. In a heat-stressed colony, returning foragers are rapidly unloaded by hive bees and this informs the other foragers that they should continue foraging (Lindauer, 1954).

Also, cavity-nesting honey-bee workers partially evacuate their nest (Dunham, 1931) at high ambient temperatures. Similarly, in open-nesting species, temperature is regulated by changes in the density of the curtain of workers that surround the nest – at high temperatures the curtain becomes very loose (Dyer and Seeley, 1991) (Fig. 12). Evacuation, and presumably large spaces between bees on an exposed comb, reduces brood nest temperature because workers avoid releasing the waste heat of metabolism within the nest. Thus, leaving the nest is more efficient than actively regulating the temperature (Dunham, 1931). The giant honey bees (*A. dorsata* and *A. laboriosa*) have an additional mechanism that may also help reduce the temperature of the nest (Seeley *et al.*, 1985; Mardan, 1989; Batra, 1996; Kastberger *et al.*, 1996; Woyke *et al.*, 2000). A large number of the bees (approx. 20% of the colony) depart from the nest for 3–5 min before returning to the nest (Kastberger *et al.*, 1996; Woyke *et al.*, 2003). On some

occasions, the departing bees synchronously defecate – possibly helping to rapidly reduce the temperature of heat-stressed insects (Mardan and Kevan, 1989). In cavity-nesting bees (*A. mellifera*), workers may shield the brood comb from high ambient temperatures by positioning themselves on hot interior regions of the nest walls (Starks and Gilley, 1999).

During the warmest part of the day, workers of the nocturnal neotropical wasp *Apoica pallens* retreat from the margins of their nest, exposing numerous cell rows. At the same time, wasps at the margins of the cluster rest with their heads inside comb cells (Hunt *et al*., 1995). Similar to the giant honey bees, hundreds of wasps may briefly depart their nest in the early evening (Hunt *et al*., 1995). This behaviour may also be related to regulating nest temperature.

Stingless bees also actively ventilate their nests; workers fan their wings within the nest while facing outwards, towards the entrance (Moritz and Crewe, 1988; Roubik, 2006). Fanning allows the nest to 'breathe', whereby air is exchanged frequently in a 'tidal' fashion. In two African species, *T. denoiti* (ground-nesting) and *T. gribodoi* (tree cavity-nesting), all air in the nest is exchanged every 1–7 h (Moritz and Crewe, 1988). Despite small entrance tubes being the only opening in some nests, fanning probably also helps regulate nest temperature (Fletcher and Crewe, 1981; Moritz and Crewe, 1988). As with some termite nests, there appears to be a trade-off between ventilation and temperature regulation in *T. denoiti*, where gas exchange is reduced during the day to prevent the nest becoming overheated. During the day, the volume of air moving in and out of a *T. denoiti* colony is considerably less than at night. Reduced air circulation during the day apparently prevents overheating, as only a small amount of fresh but hot air is drawn into the nest. However, lack of ventilation is associated with an increase in $CO_2$ levels. During the night, when the outside air is cooler, the volume of air moving through the nest is significantly greater than during the day. Therefore, $CO_2$ levels are reduced by active ventilation only at night. In contrast, in *T. gribodoi* the 'breathing' frequency is higher than in *T. denoiti*, probably because the temperatures experienced by nests are not as severe (a maximum of 36 °C outside in the shade of the tree, compared with as high as 60 °C during the day at the soil surface outside a *T. denoiti* nest entrance). Moritz and Crew interpreted this 'breathing' in both nest types as evidence for worker fanning within the nest cavity. Both species were able to maintain constant nest temperatures despite variations in ambient temperature (Moritz and Crewe, 1988). It would be interesting to test the mode of gas exchange in individuals of each species. We suggest that gas exchange mechanisms at the individual level may be in line with ventilation (by fanning) mechanisms at the colony level. Under the chthonic hypothesis for the evolution of the discontinuous gas exchange cycle (DGC) in insects, where the DGC is thought to optimize gas exchange in hypoxic and or hypercapnic environments (Lighton, 1998;

Chown *et al.*, 2006), we might expect *T. denoiti* to use the DGC and *T. gribodoi* to use a more continuous gas exchange system. In addition, it may be possible that *T. denoiti* uses the DGC during the day and a more continuous mode of gas exchange at night, similar to the colony-level variation in ventilation.

Fanning by stingless bee workers has also been reported in *S. postica* (Roubik and Peralta, 1983; Engels *et al.*, 1995), where workers fan towards and inside the entrance tube, and we have personally observed workers fanning in nests of *Asutroplebia australis*. However, like bumble bees, stingless bees apparently do not use evaporative cooling for thermoregulation; spreading of water across the cells or tongue lashing has never been reported (Engels *et al.*, 1995). For species occurring in warm climates, the nest cavity is the colony's primary means of protection from high ambient temperatures. Thus, for species like *S. postica*, which nest inside tree trunks above the understorey vegetation, exposure to extremely high temperatures is unlikely and cooling is probably unnecessary. In contrast, temperatures significantly lower than the brood nest range ($32 \pm 3\,^{\circ}C$) occur nearly every night, and therefore warming is a daily requirement for effective nest thermoregulation (Engels *et al.*, 1995).

## 4 Coordination of thermoregulation

In the previous sections, we surveyed the many active and passive mechanisms that social insect colonies use to regulate the temperature of their brood. We have described the activities of individual workers that engage in behaviour such as wing fanning or brood transportation to help regulate temperature. However, the efforts of individual workers (Table 1) would be in vain if there were no overarching mechanisms that result in a colony-level outcome of a stable brood nest temperature that is close to that required for normal development of the brood. Understanding these overarching systems is currently an area of active research (see Camazine *et al.* (2001) for a recent comprehensive review).

When we humans install an air-conditioning system, we install a control unit that monitors the temperature of the rooms and directs the refrigeration unit to turn on or off in response to the current temperature. Such a control system is completely centralized. A social insect nest, in contrast, has no such centralized control. Control is distributed among all the workers of the nest. Each worker monitors the condition of her own environment and will engage or not engage in a task, depending on her perception of the appropriate response. Key to understanding these systems of distributed control is to perceive how the simple rules followed by individual workers in response to very localized information can nonetheless result in well-regulated colony-level outcomes.

### 4.1 THE TASK THRESHOLD MODEL

Social insect nests can be regarded as 'complex systems'; those in which patterns at the global level emerge solely from many interactions among the lower-level components (Camazine *et al*., 2001). The emergent behaviour of the group of interacting agents cannot be predicted from the behaviour of any one agent, yet the group behaviour is often highly predictable from the behaviour of an average agent. In the case of social insect nests, the agents are individual workers, and the emergent 'self-organized' behaviour is the colony-level phenotype – some phenomenon like a stable brood nest temperature.

Modelling studies have shown how a colony-level outcome, such as the temperature at the centre of a swarm of honey bees or a bivouac of army ants can be precisely regulated solely by individual bees responding to their own body temperature, and without the need for communication among workers. A honey-bee swarm is a combless cluster of about 10,000 workers, a queen and a few drones. The swarm issues from a parent colony and hangs in a rugby football-shaped clump some metres from its parent colony. The swarm needs to regulate its core temperature in such a way as to conserve food reserves (contained in the stomachs of the workers), while maintaining a large number of active workers who are sufficiently warm so that they can scout for new nest sites (see Winston, 1987 for review).

Empirically, we know that workers in the centre of a honey-bee swarm maintain a temperature very close to 35 °C. These warm bees are surrounded by a mantle of cooler bees, whose temperature is about three degrees above ambient (Nagy and Stallone, 1976). Workers regulate the temperature of the swarm cluster by producing metabolic heat and by adjusting the compactness of the swarm cluster – denser when it is cold, and looser when it is warm.

Two mathematical models based on partial differential equations describing heat flux through a swarm cluster that changes density in response to temperature show that a qualitatively similar outcome to real-world swarms (stable core temperature and changing density of the cluster) can be achieved solely by workers responding to their own core temperature by adjusting their metabolic rate and closeness to their neighbours (Myerscough, 1993; Watmough and Camazine, 1995). These models demonstrate that communication among workers within the swarm is not required in order to achieve the colony-level phenotype – the outcome is entirely self-organized. Of course, these modelling results do not mean that workers in a swarm do not communicate (for example, by pheromones or sounds) to help them regulate the temperature of the swarm, only that communication and coordination is not necessary to achieve a precise core temperature. As we have no evidence that there is communication about thermoregulation within a swarm of honey bees, it seems likely that mechanisms such as those postulated by Watmough and Camazine are a good approximation of reality.

### 4.2 COORDINATION WITH COMMUNICATION

Although the models discussed above are *prima faci* evidence that communication among individual workers is not necessary to regulate the temperature in the core of a swarm of honey bees, in other systems workers *do* communicate with each other. Communication is necessary when individual workers are required to assess the needs of their colony as a whole and cannot directly make such an assessment individually. These mechanisms requiring communication are still entirely self-organized, and operate in the absence of a centralized control.

A good example of a self-organized system in which inter-individual communication is important is the means by which a honey-bee swarm selects a new nest site (Seeley and Buhrman, 1999, 2001; Britton *et al*., 2002; Seeley and Visscher, 2003, 2004; Janson *et al*., 2005). Other examples include the regulation of water collection by honey-bee colonies (see Seeley, 1995 for review).

A further example in which communication using pheromones and environmental cues is important, is the construction of termite mounds. Modelling studies, based on behavioural studies using varying levels of 'real' life parameters, have shown how following simple communication rules, such as 'I will build here because others have', can result in intricate nest mounds of large proportions. 'Architectural' differences in mound structures can emerge, not necessarily because of a change in individual behaviour, but due to changes in local environmental cues. Diversity in the landscape, often caused by previous building activities of the termites themselves or variation in pheromone levels, and differences in environmental conditions, such as wind, enable the construction of different architectural components of the nest, such as pillars or walls (Bonabeau *et al*., 1998; Ladley and Bullock, 2005). For example, evenly spaced pillars are constructed by termite builders which are attracted to 'cement pheromone' given off by recently deposited building material. A positive feedback loop is established where initially, building material is picked up, moved and deposited at random, but a tendency to deposit material where there is a high level of cement pheromone causes a concentration of deposition in certain spots, thus generating the pillars. More complex structures can arise from variations in this theme, caused by environmental cues. For example, a more open structure may be built at high temperatures when pheromones disperse rapidly.

### 4.3 THE IMPORTANCE OF INTER-INDIVIDUAL VARIABILITY

As we have seen, an effective thermoregulation system such as that which probably occurs in honey-bee swarms and army ant bivouacs can emerge from a set of simple rules followed by each individual worker (e.g.

Bonabeau *et al.* (1996)). In theory, the number of individuals involved in nest ventilation could be regulated by the simple rule: 'if air temperature is above 35 °C then fan wings'. If the temperature is high, the stimulus to act is high, whereas if the temperature is low, no worker will ventilate. Such a rule would result in a brood nest temperature that is closer to 35 °C than ambient. However, nest thermoregulation is so precise that in all probability more sophisticated systems are used to regulate the number of fanning workers.

Returning now to our air-conditioner analogy, consider a single old-fashioned wall unit with its single thermostat situated within itself. As the thermostat detects that incoming air is lower than a certain user-set temperature, it switches off the refrigerator unit. As the temperature of the incoming air rises, the thermostat then switches the refrigerator back on. Since this kind of a system can only be either on or off, and because the regulatory unit and the cooling unit are co-located, there is a strong tendency for the room temperature to oscillate around the desired temperature while rarely being at the desired temperature. A system that is more likely to achieve a stable temperature will deploy multiple thermostats distributed around the room and several refrigeration units whose output can be modulated. Such a system is capable of a graded response to temperature change: when only one thermostat achieves its threshold temperature, only one cooling unit is switched on.

We argue that intrinsic variability of workers comprising a social insect colony can be an important component of an efficient thermoregulatory system. Agent-based modelling by Myerscough and Oldroyd (2004) has shown that if all members of a social insect colony have precisely the same threshold for engaging in a task like nest heating, the emergent property of the system can be unstable. Rather, like the old-fashioned air-conditioner, the workers are either all on or all off, leading to wild oscillations in temperature around the threshold temperature (Myerscough and Oldroyd, 2004; Graham *et al.*, 2006). In contrast, modelling shows that if workers in a nest have a range of thresholds for engaging in nest thermoregulation, and these thresholds average out at the target temperature, then the colony-level outcome is much more stable than if all workers have the same threshold. This somewhat counter-intuitive outcome is analogous to the room monitored by several thermostats regulating independent cooling units.

In bumble-bee colonies, workers do indeed differ in their response thresholds for the behaviour of fanning (O'Donnell and Foster, 2001; Weidenmuller, 2004) and in other response parameters important for nest climate control. Some individuals fan every time they are exposed to a stimulus intensity exceeding their threshold, while others fan only rarely (Weidenmuller, 2004). Workers also vary in how persistently they respond to a given stimulus intensity. Weidenmuller's (2004) study provides

evidence that reinforcement is important for task specialization. The temperature threshold that would induce individual workers to commence fanning decreased across trials; thus workers with low thresholds are more likely to become specialists because their threshold will be reached more often. For bumble bees, where the queen usually mates with a single male, it is unclear what causes these differences in individual responsiveness.

In some species, especially honey bees, variance in task threshold seems to be genetically determined (reviewed in Oldroyd and Thompson, 2007) while in others, e.g. some termites whose colonies arise from a single king and queen, variance can arise from environmental factors such as larval feeding (reviewed in Oster and Wilson, 1978; Fraser *et al.*, 2000). Jones *et al.* (2004) provided empirical support for the intuitions derived from modelling: a direct link between genotypic diversity, genetically-based task specialization and an improved colony-level phenotype. First, Jones *et al.* (2004) demonstrated that genetically diverse colonies are, on average, better able to regulate the temperature of their brood nest during a short (1 h) period of extremely high temperature (40 °C) and at fluctuating ambient temperatures over a period of one week. Second, they showed that the distribution of patrilines in fanning and non-fanning workers is non-random. Third, they illustrated that the distribution of patrilines in fanning and non-fanning workers changes with ambient temperature.

## 5 Conclusions

In this review we have shown that temperature regulation in social insect nests can be remarkably precise and may involve a variety of heating and cooling mechanisms coordinated without the benefit of an individual thermo-director. We have highlighted two main issues. First, a variety of often very similar strategies for temperature regulation have evolved in different social insect groups. Some mechanisms, such as clustering and building nest structures that aid in temperature control, span many taxa. Others, such as the bizarre electro-heating pupal caps of *Vespa orientalis* appear to be unique. Second, we have explored how the simple actions of many individuals combine to form a colony-level outcome that can maintain optimal brood nest temperatures. We have given examples of the rules and mechanisms behind coordinated colony responses to temperature change in honey bees. Examples of collective thermoregulatory behaviour from other species are less well explored.

Nest thermoregulation is a useful model system for studying the mechanisms of self-organization in social insect nests, because temperature, both ambient and inside the nest, can be easily quantified and compared across species.

## References

Arias, M. C. and Sheppard, W. S. (2005). Phylogenetic relationships of honey bees (Hymenoptera: Apinae: Apini) inferred from nuclear and mitochondrial DNA sequence data. *Mol. Phyl. Evol.* **37**, 415–422.

Avitabile, D. P., Donovan, K. J. and Stafstrom, D. P. (1978). Natural nest sites of honeybee colonies in trees in Connecticut, USA. *J. Apic. Res.* **17**, 222–226.

Batra, S. W. (1996). Biology of *Apis laboriosa*, a pollinator of apples at high altitude in the Himalaya range of Garhwal, India (Hymenoptera: Apidae). *J. Kans. Entomol. Soc.* **69**, 177–181.

Bollazzi, M. and Roces, F. (2002). Thermal preference for fungus culturing and brood location by workers of the thatching grass-cutting ant *Acromyrmex heyeri*. *Ins. Soc.* **49**, 153–157.

Bonabeau, E., Deneubourg, J. and Theraulaz, G. (1996). Quantitative study of the fixed threshold model for the regulation of division of labour in insect societies. *Proc. R. Soc. Lond. B,* **263**, 1565–1569.

Bonabeau, E., Theraulaz, G., Deneubourg, J., Franks, N. R., Rafelsberger, O., Joly, J. and Blanco, S. (1998). A model for the emergence of pillars walls and the royal chamber in termite nests. *Philos. Trans. R. Soc. Lond. B,* **353**, 1561–1576.

Brian, M. V. (1963). Studies of caste differentiation in *Myrmica rubra* L. *Ins. Soc.* **2**, 91–102.

Brian, M. V. (1973). Temperature choice and its relevance to brood survival and caste determination in the ant *Myrmica rubra* L. *Physiol. Zool.* **46**, 245–252.

Britton, N. F., Franks, N. R., Pratt, S. C. and Seeley, T. D. (2002). Deciding on a new home: how do honeybees agree? *Proc. R. Soc. Lond. B,* **269**, 1383–1388.

Bujok, B., Kleinhenz, M., Fuchs, S. and Tautz, J. (2002). Hot spots in the bee hive. *Naturwissenschaften* **89**, 299–301.

Cabrera, B. J. and Kamble, S. T. (2001). Effects of decreasing thermophotoperiod on the Eastern subterranean termite (Isoptera: Rhinotermitidae). *Environ. Entomol.* **30**, 166–171.

Cabrera, B. J. and Rust, M. K. (1996). Behavioral responses to light and thermal gradients by the Western drywood termite (Isoptera: Kalotermitidae). *Environ. Entomol.* **25**, 436–445.

Camazine, S. (1991). Self-organizing pattern formation on the combs of honey bee colonies. *Behav. Ecol. Sociobiol.* **28**, 61–76.

Camazine, S., Visscher, P. K., Finley, J. and Vetter, R. S. (1999). House-hunting by honey bee swarms: collective decisions and individual behaviors. *Ins. Soc.* **46**, 348–360.

Camazine, S., Deneubourg, J., Franks, N. R., Sneyd, J., Theraulaz, G., Bonabeau, E. (2001). What is self-organization? *Self-Organization in Biological Systems*. Princeton, NJ: Princeton University Press.

Chen, Y., Hansen, L. D. and Brown, J. J. (2002). Nesting sites of the carpenter ant, *Camponotus vicinus* (Mayr) (Hymenoptera: Formicidae) in northern Idaho. *Environ. Entomol.* **31**, 1037–1042.

Chinh, T. X., Sommeijer, M. J., Boot, W. J. and Michener, C. D. (2005). Nest and colony characteristics of three stingless bee species in Vietnam with the first description of the nest of *Listotrigona carpenteri* (Hymenoptera: Apidae: Meliponini). *J. Kans. Entomol. Soc.* **78**, 363–372.

Chown, S. L., Gibbs, A. G., Hetz, S. K., Klok, C. J., Lighton, J. R. B. and Marias, E. (2006). Discontinuous gas exchange in insects: a clarification of hypotheses and approaches. *Physiol. Biochem. Zool.* **79**, 333–343.

Cole, B. J. (1994). Nest architecture in the Western harvester ant, *Pogonomyrmx occidentalis* (Cresson). *Ins. Soc.* **41**, 401–410.
Darlington, J. P. E. C. (1984). Two types of mound built by the termite *Macrotermes subhyalinus* in Kenya. *Insect Sci. Appl.* **5**, 481–492.
Doedikar, G. B., Ghatge, A. I., Phadke, R. P., Mahindre, D. B., Kshirsager, K. K., Muvel, K. S. and Thaker, C. V. (1977). Nesting behaviour of Indian honeybees III. Nesting behaviour of *Apis dorsata* Fab. *Indian Bee Journal* **39**, 1–12.
Dunham, W. E. (1931). A colony of bees exposed to high external temperatures. *J. Econ. Entomol.* **24**, 606–611.
Dyer, F. C. and Seeley, T. D. (1991). Nesting behavior and the evolution of worker tempo in four honey bee species. *Ecology* **72**, 156–170.
Elton, C. (1932). Orientation of the nests of the *Formica truncorum* F. in north Norway. *J. Anim. Ecol.* **1**, 192–193.
Engel, M. S. and Schultz, T. R. (1997). Phylogeny and behavior in honey bees (Hymenoptera: Apidae). *Ann. Entomol. Soc. Am.* **90**, 43–53.
Engels, W., Rosenkranz, P. and Engels, E. (1995). Thermoregulation in the nest of the neotropical stingless bee *Scaptotrigona postica* and a hypothesis on the evolution of the temperature homeostasis in highly eusocial bees. *Stud. Neotropical Fauna and Environ.* **30**, 193–205.
Fahrenholz, L., Lamprecht, I. and Schricker, B. (1989). Thermal investigation of a honey bee colony: thermoregulation of the hive during summer and winter and heat production of members of different bee castes. *J. Comp. Physiol. B,* **159**, 551–560.
Fletcher, D. J. C. and Crewe, R. M. (1981). Nest structure and thermoregulation in the stingless bee *Trigona (Plebeina) denoiti* Vachal (Hymenoptera: Apidae). *J. Entomol. Soc. South Africa* **44**, 183–196.
Franck, P., Cameron, E., Good, G., Rasplus, J. Y. and Oldroyd, B. P. (2004). Nest architecture and genetic differentiation in a species complex of Australian stingless bees. *Mol. Ecol.* **13**, 2317–2331.
Franks, N. R. (1989). Thermoregulation in army ant bivouacs. *Physiol. Entomol.* **14**, 397–404.
Fraser, V. S., Kaufman, B., Oldroyd, B. P. and Crozier, R. H. (2000). Genetic influence on caste allocation in the ant *Camponotus consobrinus*. *Behav. Ecol. Sociobiol.* **47**, 188–194.
Free, J. B. and Simpson, J. (1963). The respiratory metabolism of honey-bee colonies at low temperatures. *Entomol. Exp. App.* **6**, 234–238.
Frouz, J. (2000). The effect of nest moisture on daily temperature regime in the nests of *Formica polyctena* wood ants. *Ins. Soc.* **47**, 229–235.
Gibo, D. L., Dew, H. E. and Hajduk, A. S. (1974b). Thermoregulation in colonies of *Vespula arenaria* and *Vespula maculata* (Hymenoptera: Vespidae) II. The relation between colony biomass and calorie production. *Can. Entomol.* **106**, 873–879.
Gibo, D. L., Temporale, A., Lamarre, T. P., Soutar, B. M. and Dew, H. E. (1977). Thermoregulation in colonies of *Vespula arenaria* and *Vespula maculata* (Hymenoptera: Vespidae) III. Heat production in queen nests. *Can. Entomol.* **109**, 615–620.
Gibo, D. L., Yarascavitch, R. M. and Dew, H. E. (1974a). Thermoregulation in colonies of *Vespula arenaria* and *Vespula maculata* (Hymenoptera: Vespidae) under normal conditions and under cold stress. *Can. Entomol.* **106**, 503–507.
Graham, S., Myerscough, M. R., Jones, J. C. and Oldroyd, B. P. (2006). Modelling the role of intracolonial genetic diversity on regulation of brood temperature in honey bee (*Apis mellifera* L.) colonies. *Ins. Soc.* **53**, 226–232.

Greaves, T. (1964). Temperature studies of termite colonies in living trees. *Aust. J. Zool.* **12**, 250–262.
Grigg, G. C. and Underwood, A. J. (1977). An analysis of the orientation of 'magnetic' termite mounds. *Aust. J. Zool.* **25**, 87–94.
Hasselrot, T. B. (1960). Studies on Swedish bumblebees (Genus *Bombus* Latr.): their domestication and biology. *Opusc. Entomol. Supplementum,* **17**, 1–192.
Hazelhoff, E. H. (1954). Ventilation in a bee-hive during summer. *Physiol. Comp. Oecol.* **3**, 343–364.
Heinrich, B. (1972). Patterns of endothermy in bumble bee queens, drones and workers. *J. Comp. Physiol.* **77**, 65–79.
Heinrich, B. (1974a). Thermoregulation in bumblebees. I. Brood incubation by *Bombus vosnesenskii* queens. *J. Comp. Physiol.* **88**, 129–140.
Heinrich, B. (1974b). Pheromone induced brooding behavior in *Bombus vosnesenskii* and *B. edwardsii* (Hymenoptera: Bombidae). *J. Kans. Entomol. Soc.* **47**, 396–404.
Heinrich, B. (1979). *Bumblebee Economics*. Cambridge, MA: Harvard University Press.
Heinrich, B. and Kammer, A. (1973). Activation of the fibrillar muscles of the bumblebee during warm-up stabilization of thoracic temperature, and flight. *J. Exp. Biol.* **58**, 677–688.
Himmer, A. (1927). Ein Beitrag zur Kenntnis des Warmehaushalts im Nestbau sozialer Hautfluger. *J. Comp. Physiol. A* **5**, 375–389.
Himmer, A. (1932). Die Temperaturverhaltnisse bei den sozialen. *Biol. Rev.* **7**, 224–253.
Hölldobler, B. and Wilson, E. O. (1990). *The Ants*. Cambridge, MA: Harvard University Press.
Hozumi, S. and Yamane, S. (2001). Incubation ability of the functional envelope in paper wasp nest (Hymenoptera, Vespidae, *Polistes*): I. Field measurements of nest temperature using paper models. *J. Ethol.* **19**, 39–46.
Hubbard, M. D. and Cunningham, W. G. (1977). Orientation of mounds of the ant *Solenopsis invicta* (Hymenoptera, Formicidae, Myrmicinae). *Ins. Soc.* **24**, 3–7.
Hunt, J. H., Jeanne, R. L. and Keeping, M. (1995). Observation on *Apoica pallens*, a nocturnal Neotropical social wasp (Hymenoptera: Vespidae, Polistinae, Epiponini). *Ins. Soc.* **42**, 223–236.
Ishay, J. S. (1973). Thermoregulation by social wasps: behavior and pheromones. *Trans. N. Y. Acad. Sci.* **35**, 447–462.
Ishay, J. S. and Barenholz-Paniry, V. (1995). Thermoelectric effect in hornet (*Vespa orientalis*) silk and thermoregulation in a hornet's nest. *J. Insect Physiol.* **41**, 753–759.
Jacklyn, P. M. (1992). ''Magnetic'' termite mound surfaces are oriented to suit wind and shade conditions. *Oecologia* **91**, 385–395.
Jackson, W. B. (1957). Microclimate patterns in the army ant bivouac. *Ecology* **38**, 276–285.
Janson, S., Middendorf, M. and Beekman, M. (2005). Honeybee swarms: how do scouts guide a swarm of uninformed bees? *Anim. Behav.* **70**, 349–358.
Jay, S. C. (1963). The development of honeybees in their cells. *J. Apic. Res.* **2**, 117–134.
Jaycox, E. R. and Parise, S. G. (1980). Homesite selection by Italian honey bee swarms, *Apis mellifera ligustica* (Hymenoptera: Apidae). *J. Kans. Entomol. Soc.* **53**, 171–178.
Jaycox, E. R. and Parise, S. G. (1981). Homesite selection by swarms of black-bodied honey bees, *Apis mellifera caucasica* and *A. m. carnica* (Hymenoptera: Apidae). *J. Kans. Entomol. Soc.* **54**, 697–703.
Jeanne, R. L. and Morgan, R. C. (1992). The influence of temperature on nest site choice and reproductive strategy in a temperate zone *Polistes* wasp. *Ecol. Entomol.* **17**, 135–141.

Jones, J. C., Helliwell, P., Beekman, M., Maleszka, R. and Oldroyd, B. P. (2005). The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. *J. Comp. Physiol. A* **191**, 1121–1129.
Jones, J. C., Myerscough, M. R., Graham, S. and Oldroyd, B. P. (2004). Honey bee nest thermoregulation: diversity promotes stability. *Science* **305**, 402–404.
Joseph, Z. and Ishay, J. S. (2004). Silk structure in the hornet cocoon. *J. Electron Microsc.* **53**, 293–304.
Kastberger, G., Winder, O., Hoetzl, T. and Raspotnig, G. (1996). Behavioral features of a periodic form of massed flight activity in the giant honey bee *Apis dorsata*. *Apidologie* **27**, 381–395.
Kleinhenz, M., Bujok, B., Fuchs, S. and Tautz, J. (2003). Hot bees in empty broodnest cells: heating from within. *J. Exp. Biol.* **206**, 4217–4231.
Korb, J. (2003). Thermoregulation and ventilation of termite mounds. *Naturwissenschaften* **90**, 212–219.
Korb, J. and Linsenmair, K. (1998a). Experimental heating of *Macrotermes bellicosus* (Isoptera, Macrotermitinae) mounds: what role does microclimate play in influencing mound architecture? *Ins. Soc.* **45**, 335–342.
Korb, J. and Linsenmair, K. (1998b). The effects of temperature on the architecture and distribution of *Macrotermes bellicosus* (Isoptera, Macroterminae) mounds in different habitats of a West African Guinea savanna. *Ins. Soc.* **45**, 51–65.
Korb, J. and Linsenmair, K. (1999). The architecture of termite mounds: a result of a trade-off between thermoregulation and gas exchange? *Behav. Ecol.* **10**, 312–316.
Korb, J. and Linsenmair, K. (2000a). Thermoregulation of termite mounds: what role does ambient temperature and metabolism of the colony play? *Ins. Soc.* **47**, 357–363.
Korb, J. and Linsenmair, K. (2000b). Ventilation of termite mounds: new results require a new model. *Behav. Ecol.* **11**, 486–494.
Kronenberg, F. and Heller, H. C. (1982). Colonial thermoregulation in honey bees (*Apis mellifera*). *J. Comp. Physiol. B,* **148**, 65–76.
Kuriachan, I. and Vinson, S. B. (2000). A queen's worker attractiveness influences her movement in polygynous colonies of the red imported fire ant (Hymenoptera: Formicidae) in response to adverse temperature. *Environ. Entomol.* **29**, 943–949.
Ladley, D. and Bullock, S. (2005). The role of logistic constraints in termite construction of chambers and tunnels. *J. Theor. Biol.* **234**, 551–564.
Lapointe, S. L., Serrano, M. S. and Jones, P. G. (1998). Microgeographic and vertical distribution of *Acromyrmex landolti* (Hymenoptera: Formicidae) nests in a neotropical savanna. *Environ. Entomol.* **27**, 636–641.
Lighton, J. R. B. (1998). Notes from underground: towards ultimate hypotheses of cyclic discontinuous gas-exchange in Tracheate Arthropods. *Am. Zool.* **38**, 483–491.
Lindauer, M. (1954). Temperaturregulierung und Wasserhaushalt im Bienenstaat. *J. Comp. Physiol. A,* **36**, 391–432.
Lindauer, M. (1955). Schwarmbienen auf Wohnungssuche. *J. Comp. Physiol. A,* **37**, 263–324.
Luscher, M. (1961). Air-conditioned termite nests. *Sci. Am.* **205**, 138–145.
Makino, S. and Yamane, S. (1980). Heat production by the foundress of *Vespa simillima*, with description of its embryo nest (Hymenoptera: Vespidae). *Insecta Matsumurana* **19**, 89–101.
Mardan, M. B. (1989). Thermoregulation in the Asiatic giant honeybee *Apis dorsata* (Hymenoptera: Apidae). Ph.D. dissertation, University of Guelph, Ontario.
Mardan, M. B. and Kevan, P. G. (1989). Honeybees and yellow rain. *Nature* **341**, 191.
Martin, S. J. (1988). Thermoregulation in *Vespa simillima xanthoptera* (Hymenoptera, Vespidae). *Kontyu* **56**, 674–677.

Martin, S. J. (1992). Nest thermoregulation in *Vespa affinis* (Hymenoptera, vespidae). *Jpn. J. Entomol* **60**, 483–486.
McMullan, J. B. and Brown, M. J. F. (2005). Brood pupation temperature affects the susceptibility of honeybees (*Apis mellifera*) to infestation by tracheal mites (*Acarapis woodi*). *Apidologie* **36**, 97–105.
Miyata, H., Shimamura, T., Hirosawa, H. and Higashi, S. (2003). Morphology and phenology of the primitive ponerine army ant *Onychomyrmex hedleyi* (Hymenoptera: Formicidae: Ponerinae) in a highland rainforest of Australia. *J. Nat. Hist.* **37**, 115–125.
Moritz, R. F. A. and Crewe, R. M. (1988). Air ventilation in nests of two African stingless bees *Trigona denoiti* and *Trigona gribodoi*. *Experientia* **44**, 1024–1027.
Moritz, R. F. A. and Southwick, E. E. (1992). *Bees as Superorganisms*. Berlin: Springer.
Myerscough, M. R. (1993). A simple model for temperature regulation in honeybee swarms. *J. Theor. Biol.* **162**, 381–393.
Myerscough, M. R. and Oldroyd, B. P. (2004). Simulation models of the role of genetic variability in social insect task allocation. *Ins. Soc.* **51**, 146–152.
Nagy, K. A. and Stallone, J. N. (1976). Temperature maintenance and CO2 concentration in a swarm cluster of honey bees, *Apis mellifera*. *Comp. Biol. Physiol. A,* **55**, 169–171.
Navarro, J. G. and Jaffe, K. (1985). On the adaptive value of nest features in the grass-cutting ant *Acromyrmex landolti*. *Biotropica* **17**, 347–348.
O'Donnell, S. and Foster, R. L. (2001). Thresholds of response in nest thermoregulation by worker bumble bees, *Bombus bifarius nearcticus* (Hymenoptera: Apidae). *Ethology* **107**, 387–399.
Ofer, J. (1970). *Polyrhachis simplex* the weaver ant of Israel. *Ins. Soc.* **17**, 49–82.
Oldroyd, B. P., Sylvester, H. A., Wongsiri, S. and Rinderer, T. E. (1994). Task specialization in a wild bee, *Apis florea* (Hymenoptera: Apidae), revealed by RFLP banding. *Behav. Ecol. Sociobiol.* **34**, 25–30.
Oldroyd, B. P. and Wongsiri, S. (2006). *Asian Honey Bees Biology, Conservation and Human Interactions*. Cambridge, MA: Harvard University Press.
Oldroyd, B. P. and Thompson, G. J. (2007). Behavioural genetics of the honey bee, *Apis. Adv. Ins. Physiol.* **33**, 1–49.
Oster, G. F. and Wilson, E. O. (1978). *Caste and Ecology in the Social Insects*. Princeton, NJ: Princeton University Press.
Powell, R. J. and Stradling, D. J. (1986). Factors influencing the growth of *Attamyces bromatificus*, a symbiont of attine ants. *Trans. Br. Mycol. Soc.* **87**, 205–213.
Pranschke, A. M. and Hooper-Bui, L. M. (2003). Influence of abiotic factors on red imported fire ant (Hymenoptera: Formicidae) mound population ratings in Louisiana. *Environ. Entomol.* **32**, 204–207.
Reddy, S. M. and Reddy, C. C. (1993). Studies on the distribution of nests of giant honey bee (*Apis dorsata* F.). *Indian Bee Journal* **55**, 36–39.
Riabinin, K., Kozhevnikov, M. and Ishay, J. S. (2004). Ventilating activity at the hornet nest entrance. *J. Ethol.* **22**, 49–53.
Rinderer, T. E., Collins, A. M., Bolten, A. B. and Harbo, J. R. (1981). Size of nest cavities selected by swarms of Africanized honeybees in Venezuela. *J. Apic. Res.* **20**, 160–164.
Rinderer, T. E., Tucker, K. W. and Collins, A. M. (1982). Nest cavity selection by swarms of European and Africanized honey bees. *J. Apic. Res.* **21**, 98–103.
Roces, F. and Kleineidam, C. (2000). Humidity preference for fungus culturing by workers of the leaf-cutting ant *Atta sexdens rubropilosa*. *Ins. Soc.* **47**, 348–350.

Roces, F. and Nunez, J. A. (1989). Brood translocation and circadian variation of temperature preference in the ant *Camponotus mus*. *Oecologia* **81**, 33–37.
Roces, F. and Nunez, J. A. (1995). Thermal sensitivity during brood care in workers of two *Camponotus* ant species: circadian variation and its ecological correlates. *J. Insect Physiol.* **41**, 659–669.
Rosengren, R., Fortelius, W., Lindstrom, K. and Luther, A. (1987). Phenology and causation of nest heating and thermoregulation in red wood ants of the *Formica rufa* group studied in coniferous forest habitats in southern Finland. *Ann. Zool. Fenn.* **24**, 147–155.
Roubik, D. W. (2006). Stingless bee nesting biology. *Apidologie* **37**, 1–20.
Roubik, D. W. and Peralta, F. J. A. (1983). Thermodynamics in nests of two Melipona species in Brasil. *Acta Amazon.* **13**, 453–466.
Ruttner, F. (1988). *Biogeography and Taxonomy of Honeybees*. Berlin: Springer.
Sakagami, S. F. (1960). Preliminary report of the specific difference of behavior and other ecological characters between European and Japanese honeybees. *Acta Hymenopterol.* **1**, 171–198.
Scherba, G. (1958). Reproduction, nest orientation and population structure of an aggregation of mound nests of *Formica ulkei* Emery (Formicidae). *Ins. Soc.* **5**, 201–213.
Schmidt, J. O. and Hurley, R. (1995). Selection of nest cavities by Africanized and European honey bees. *Apidologie* **26**, 467–475.
Schneirla, T. C., Brown, R. Z. and Brown, F. C. (1954). The bivouac or temporary nest as an adaptive factor in certain terrestrial species of army ants. *Ecol. Monogr.* **24**, 269–296.
Seeley, T. D. (1976). The nest of the honey bee (*Apis mellifera* L.). *Ins. Soc.* **23**, 495–512.
Seeley, T. D. (1977). Measurement of nest cavity volume by the honey bee (*Apis mellifera*). *Behav. Ecol. Sociobiol.* **2**, 202–227.
Seeley, T. D. (1985). *Honeybee Ecology*. Princeton, NJ: Princeton University Press.
Seeley, T. D. (1995). *The Wisdom of the Hive*. Cambridge, MA: Harvard University Press.
Seeley, T. D. and Morse, R. A. (1978). Nest site selection by the honey bee, *Apis mellifera*. *Ins. Soc.* **25**, 323–337.
Seeley, T. D. and Heinrich, B. (1981). Regulation of temperature in the nests of social insects. In: *Insect Thermoregulation* (ed. Heinrich, B.), pp. 159–234. New York: Wiley.
Seeley, T. D., Seeley, R. H. and Akratanakul, P. (1982). Colony defence strategies of the honeybees in Thailand. *Ecol. Monogr.* **52**, 43–63.
Seeley, T. D., Nowicke, J. W., Meleson, M., Guillemin, J. and Akratanakul, P. (1985). Yellow rain. *Sci. Am.* **253**, 128–137.
Seeley, T. D. and Buhrman, S. C. (1999). Group decision making in swarms of honey bees. *Behav. Ecol. Sociobiol.* **45**, 19–31.
Seeley, T. D. and Buhrman, S. C. (2001). Nest-site selection in honey bees: how well do swarms implement the 'best-of-N' decision rule. *Behav. Ecol. Sociobiol.* **49**, 416–427.
Seeley, T. D. and Visscher, P. K. (2003). Choosing a home: how the scouts in a honey bee swarm perceive the completion of their group decision making. *Behav. Ecol. Sociobiol.* **54**, 511–520.
Seeley, T. D. and Visscher, P. K. (2004). Group decision making in nest-site selection by honey bees. *Apidologie* **35**, 101–116.
Stabentheiner, A., Pressl, H., Papst, T., Hrassnigg, N. and Crailsheim, K. (2003). Endothermic heat production in honeybee winter clusters. *J. Exp. Biol.* **206**, 353–358.

Starks, P. T. and Gilley, D. C. (1999). Heat shielding: a novel method of colonial thermoregulation in honey bees. *Naturwissenschaften* **86**, 438–440.
Tautz, J., Maier, S., Groh, C., Rossler, W. and Brockmann, A. (2003). Behavioural performance in adult honey bees is influenced by the temperature experienced during their pupal development. *Proc. Natl. Acad. Sci. USA* **100**, 7343–7347.
Thapa, R. and Wongsiri, S. (1994). Distinct fanning behaviour of two dwarf honeybees *Apis andreniformis* (Smith) and *Apis florea* (Fab.). In: *Second International Conference of the Asian Apicultural Association* (ed. Sakai, T.), pp. 344–345. Yogyakata.
Tschinkel, W. R. (2006). *The Fire Ants*. Cambridge, MA: Belknap Press.
Turner, J. S. (1994). Ventilation and thermal constancy of a colony of a southern African termite (*Odontotermes transvaalensis*: Macrotermitinae). *J. Arid. Environ.* **28**, 231–248.
Turner, S. J. (2001). On the mound of *Macrotermes michaelseni* as an organ of respiratory gas exchange. *Physiol. Biochem. Zool.* **74**, 798–822.
Underwood, B. A. (1986). The natural history of *Apis laboriosa* in Nepal. MSc thesis, Cornell University.
Underwood, B. A. (1990). Seasonal nesting cycle and migration patterns of the Himalayan honey bee *Apis laboriosa*. *Natl. Geogr. Res.* **6**, 276–290.
Watmough, J. and Camazine, S. (1995). Self-organized thermoregulation of honeybee clusters. *J. Theor. Biol.* **176**, 391–402.
Weber, N. A. (1957). Dry season adaptations of fungus growing ants and their fungi. *Anat. Rec.* **128**, 638.
Weidenmuller, A. (2004). The control of nest climate in bumblebee (*Bombus terrestris*) colonies: interindividual variability and self reinforcement in fanning response. *Behav. Ecol.* **15**, 120–128.
Weir, J. S. (1973). Air flow, evaporation and mineral accumulation in mounds of *Macrotermes subhyalinus* (Rambur). *J. Anim. Ecol.* **42**, 509–520.
Wille, A. and Michener, C. D. (1973). The nest architecture of stingless bees with special reference to those of Costa Rica (Hymenoptera: Apidae). *Rev. Biol. Trop.* **21**, 1–278.
Wilson, E. O. (1971). *The Insect Societies*. Cambridge, MA: Harvard University Press.
Winston, M. L. (1987). *The Biology of the Honey Bee*. Cambridge, MA: Harvard University Press.
Wongsiri, S., Lekprayoon, R., Thapa, A., Thirakupt, K., Rinderer, T. E., Sylvester, H. A., Oldroyd, B. P. and Boocham, U. (1997). Comparative biology of *Apis andreniformis* and *Apis florea* in Thailand. *Bee World* **78**, 23–35.
Woyke, J., Wilde, J. and Wilde, M. (2000). Swarming, migrating and absconding of Apis dorsata colonies. In: *Seventh International Conference on tropical bees: Management and Diversity and Fifth Asian Apicultural Association Conference* (ed. Wongsiri, S.), pp. 183–188. Cardiff: International Bee Research Association.
Woyke, J., Wilde, J. and Wilde, M. (2003). Periodic mass flight of *Apis laboriosa* in Nepal. *Apidologie* **34**, 121–127.
Yamane, S. (1988). A preliminary note of the thermal property in two different types of *Ropalidia* combs (Hymenoptera, Vespidae). *Kontyu* **56**, 896–900.
Yamane, S. and Kawamichi, T. (1975). Bionomic comparisons of *Polistes biglumis* (Hymenoptera, Vespidae) at two different localities in Hokkaido, northern Japan, with reference to its probable adaptation to cold climate. *Kontyu* **43**, 214–232.

# The Organule Concept of Insect Sense Organs: Sensory Transduction and Organule Evolution

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## 1 Introduction

Vincent Dethier, in the preface to his 1963 book "The Physiology of Insect Senses" nominated the period between the 1930s and 1960s as the era in which technical advances in electrophysiology and electron microscopy vastly increased our knowledge of sensory physiology. Continuing through the 1960s and 1970s, transmission electron microscopy helped elucidate the

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relationships of the cells making up the sense organ and provided morphological clues to their functional mechanisms. Once the scope of sense organ diversity was recognized and structural characteristics of the main modalities defined, the rate of growth in our knowledge of how they functioned slowed. At around the same time, the field of molecular genetics using the model organism *Drosophila melanogaster* picked up the baton and the sensory system once again became a major focus of attention. The primary reason for this was that understanding the developmental genetics of the peripheral nervous system (PNS) of *Drosophila* was seen as a way of understanding the development of the brain, providing insights into cell lineage determination and the genetic basis of cell identities. Most often, *Drosophila* is compared with the nematode *C. elegans*, and both are compared with vertebrates, reflecting the current genetic, developmental and physiological focus on model organisms. On the other hand, a wealth of useful morphological information is available for a broad range of insects and, to a lesser extent, other arthropods; but the comparative approach has been under-utilized.

The developmental approach to sense organ morphology using the *Drosophila* paradigm has revealed a tremendous amount about how variations between sense organs of different modalities arise. An example is the origin of multidendritic (or type II) neurons. In the late 1990s, lineage tracing and mutant analysis revealed that this class of sensory neurons is derived from lineages that give rise to external sensilla (Brewster and Bodmer, 1995; Vervoort *et al.*, 1997), immediately changing our perspective on their function and raising many questions about their evolution. This origin of multidendritic neurons appears to be evolutionarily conserved rather than *Drosophila*-specific, as similar mechanisms and indeed homologous multidendritic neurons were seen in caterpillars (Grueber and Truman, 1999). Furthermore, multidendritic neurons have been identified as the origin of a response to noxious stimuli: the first identification of specialized insect nociceptors (Tracey *et al.*, 2003).

These *Drosophila* breakthroughs built upon the insightful studies of sense organ development carried out on insects such as the blood-sucking bug *Rhodnius* by Sir Vincent Wigglesworth and the milkweed bug *Oncopeltus* by his student Peter Lawrence (see Edwards, 1998).

The term *organule* was coined by Lawrence (1966) as comparable to the German term Kleineorgan (Henke, 1953). It was used to describe epidermal structures that are formed by a small number of intimately associated, clustered cells, too small in number to be regarded as full-fledged organs but sufficiently complex and self-contained to be regarded as mini-organs. Sensilla, dermal glands, oenocytes and scales were included in this category. While the term was not intended to imply that different organules are homologous, i.e. derived from a common ancestral multicelled unit, evidence for homology among these organules will be investigated in this review, with the

hope that the evolutionary and developmental flexibility of the organule will become apparent.

In recent years, genomic and genetic approaches, particularly using *Drosophila*, have provided new insights into the genes and proteins involved in mechanoreception, thermoreception, chemoreception, and nociception. A further aim of this review is to summarize our rapidly expanding knowledge of sensory transduction in two major classes of sense organs, mechanoreceptors and chemoreceptors. My main desire is to take this molecular and genetic information and place it in a morphological and evolutionary context, focussing on what we know about the relationships and structures of the cells making up the sense organs.

The use of *Drosophila* as the genetic model has unavoidably led to a bias toward treating it as the canonical insect. Insects have an ancient and diverse lineage, and *Drosophila* represents one twig of a highly branched tree. Compared to the insect groundplan, advanced flies have an unusual way of developing, with the number of larval moults reduced, the larval head being drawn into the body during embryogenesis, reduction of the larval sensory system, growth through polyploidy of the hypodermal cells rather than cell division, a most extreme form of holometaboly whereby the developing adult head, legs and wings lie wholly within the larval body, and loss of most of the larval body at metamorphosis and its replacement with imaginal disc-derived tissues in the pupa (Merritt, 2005). Therefore, it must be kept in mind that insights from *Drosophila* are insights into the developmental mechanisms of a specialized group of advanced flies. Ultimately, the emergence of model insects that span the breadth of insect diversity will tell us more about how the insect nervous system has evolved.

## 2 Sensory modalities

Sense organs can be considered under the following main modalities: vision, mechanoreception, gustation, olfaction, hygroreception and thermoreception. In this review, I will not be concerned with the development of photoreceptors and phototransduction cascades, but I will discuss some of the evolutionary similarities they bear to sensilla. The structure and function of insect sensory receptors have been reviewed: mechanoreceptors (Keil and Steinbrecht, 1984; Field and Matheson, 1998), chemoreceptors (Slifer, 1970; Zacharuk, 1980), olfactory receptors (Keil and Steinbrecht, 1984), and thermo/hygroreceptors (Altner and Prillinger, 1980; Altner and Loftus, 1985).

The ciliated neurons associated with sensilla (Fig. 1A), classically known as type I neurons, are distinctively different from type II neurons, which are multidendritic and without a cilium in any of the dendrites. Moreover, type II neurons tend to have widely dispersed dendritic arbors lying beneath the hypodermis or in association with support structures (Fig. 1B).

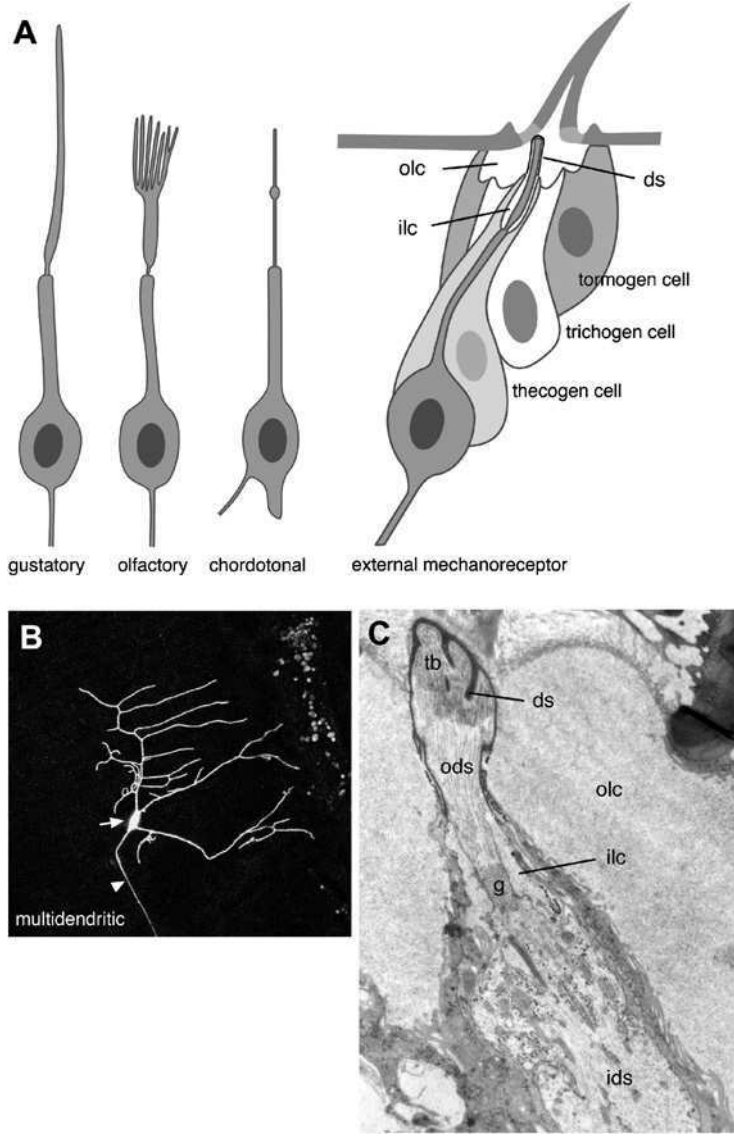



FIG. 1 (A) Diagram of typical type I neurons. The external mechanoreceptor shows the relationships of support cells and the location of the inner (ilc) and outer (olc) lymph cavities. (B) A type II (multidendritic) neuron in a *Drosophila* larva. The soma is indicated with an arrow and the axon with an arrowhead. (C) Transmission electron micrograph of the dendrite of an external mechanoreceptor in longitudinal section. The tubular body (tb) is surrounded by a dendritic sheath (ds). The inner (ids) and outer (ods) dendritic segments are separated by a ciliary structure that typically contains granular material (g) in this species, the blowfly *Lucilia cuprina*. (B) is modified from Williams and Truman (2004), with permission. (C) is modified from Merritt (1989), with permission.

## 3 Mechanoreceptors

There are three types of mechanoreceptors in insects. Two are of type I neurons, with monopolar, ciliated dendrites. They include: (1) external sensilla (es) such as hair plates, tactile hairs and campaniform sensilla; and (2) chordotonal sensilla (ch) that are found in various locations on the body. The third category is multidendritic (type II) neurons (Fig. 1). Type I sense organs, the es and ch, are obviously homologous organules with similar lineages and comparable cell identities (Merritt, 1997; Lai and Orgogozo, 2004; Hartenstein, 2005).

In insects, a typical external mechanoreceptor is innervated by a single sensory neuron that gives rise to a single dendrite and axon at opposite poles of the soma (Fig. 1A). The neural soma and part of the dendrite are surrounded by a thecogen cell that intimately binds to the inner dendritic segment. It forms an extracellular space, the inner lymph cavity, around the cilium and secretes an extracellular dendritic sheath around the distal dendrite. The dendrite contains a short ciliary derivative, the cilium, with nine pairs of ciliary doublets and no central pair ($9 \times 2 + 0$), that divides the dendrite into an inner and outer dendritic segment (Fig. 1C). The dendrite terminates internally at the base of a cuticular hair or cuticular campaniform dome, remaining surrounded by the dendritic sheath (Keil and Steinbrecht, 1984; Keil, 1997a).

Early ultrastructural studies recognized an iconic attribute of the external mechanoreceptors: the presence of a dense microtubule-based body (termed tubular body) within the tip of the dendrite (Thurm, 1964). From the time of its initial discovery, the tubular body has been recognized as the likely site for sensory transduction. Its location at the tip of the dendrite, where it forms a dense cytoskeleton, indicates that it could be compressed by hair movements (Thurm, 1964). The generation of the receptor potential was suspected to be due to mechanically activated ion channels in the membrane stretched over the tubular body (Rice *et al*., 1973) or mechanically activated by membrane-integrated cones that link the microtubules of the tubular body and ion channels in the membrane (Keil and Steinbrecht, 1984). Direct activation of the ion channels by mechanical force is likely because of the very short (200 μs) latencies between stimulus and the resulting current (Walker *et al*., 2000).

Other ideas of sensory transduction in chordotonal and external mechanoreceptors have been based upon the similarities between the axoneme of sensory dendrites and flagella, suggesting that microtubules are involved in mechanical transduction (Crouau, 1983). These ideas, reviewed by French (1988), have been largely discounted, but the demonstration of active movement of the antennal segment by forces generated in ch, demonstrated by Gopfert and co-workers, has called for a re-evaluation, especially in chordotonal organs (see Section 3.2.4).

Chordotonal sensilla usually have 1–3 sensory neurons. The neuron is characterized by a long, straight cilium lying in a scolopale lumen surrounded by a heavily developed cytoskeleton of scolopale rods within the scolopale cell. There are two types of ch, referred to classically as mononematic and amphinematic ch (Moulins, 1976; Field and Matheson, 1998; Yack, 2004). Mononematic ch have no direct contact with the cuticle, and the dendritic sheath is present in the form of a compact, extracellular dome (Fig. 2). Amphinematic ch organs retain a close association with the cuticle and have from 2 to 3 neurons. Where three neurons are present, one of them has an expanded, outer dendritic segment (Moulins, 1976; Field and Matheson, 1998). In *Drosophila*, the cell complement and development of the mononematic ch is well understood. They comply with the canonical

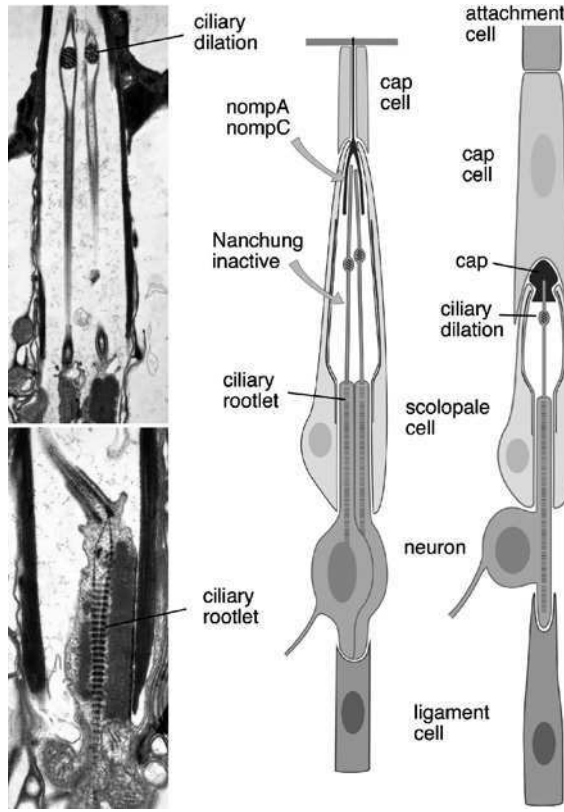



FIG. 2 Left. Transmission electron micrographs of the ciliary region of chordotonal sensilla of Johnston's organ in *Drosophila*. Courtesy of D. Abel and D. Eberl, University of Iowa, Iowa, USA. Right. Diagram of an amphinematic chordotonal sensillum with two sensory neurons, typical of an antennal chordotonal organ of *Drosophila*. Far right. A typical mononematic chordotonal sensillum with a single neuron, based on a larval body wall chordotonal organ (Merritt, 1997).

five-cell lineage (see Section 6), and utilize EGF receptor recruitment mechanisms to induce the formation of additional ligament attachment cells (Inbal *et al*., 2004) and oenocytes (Elstob *et al*., 2001; Rusten *et al*., 2001). The lineage and identity of component cells of the Johnston's organ amphinematic sensilla are less well known (Caldwell and Eberl, 2002; Boekhoff-Falk, 2005).

Chordotonal organs share a number of features with external mechanoreceptors, however, there are some pronounced differences based upon the degree of development of particular structures (Moulins, 1976; French, 1988; Merritt, 1997). Some of the most substantial differences are: (1) highly developed, actin-rich scolopale within the scolopale cell surrounding the dendrite, (2) high degree of development of the ciliary rootlets, and (3) the dendrite has an extended ciliary region compared to external mechanoreceptors and the distal tip is either embedded in a dendritic cap or closely applied to a dendritic sheath. Based purely upon observations at the ultrastructural level, it appears that many of the structural attributes of chordotonal organs are designed to hold the dendrite taut within the scolopale structures, pointing to the extended ciliary region as the site of sensory transduction (Field and Matheson, 1998).

### 3.1 LARVAL TOUCH INSENSITIVITY, UNCOORDINATED ADULTS AND DEAFNESS

In a search for genes associated with touch perception, Kernan *et al*. (1994) screened mutagenized flies for tactile insensitivity by selecting larvae that failed to turn or withdraw in response to a light stroke with an eyelash. To characterize the sensory defects, the screens were followed up by electrophysiological analyses of the receptors, with examination at the light microscope or electron microscope level for structural defects and, where possible, isolation of the genes and localization of their expression. A number of mutations were isolated, namely, *uncoordinated* (*unc*), *uncoordinated-like* (*uncl*) and *touch-insensitive larva B* (*tilB*) (Kernan *et al*., 1994). Extracellular recording of trans-epithelial potential (TEP) was measured as was the mechanoreceptor potential (MRP) of tactile hairs. Normal TEP values are taken to indicate that the support cells are capable of maintaining the appropriate ionic milieu within the outer lymph cavity and the MRP is a rapid negative deflection of the resting potential in response to hair movement. *Unc* and *uncl* displayed a normal TEP but the MRP was absent, indicating that the generation of the receptor potential is affected; whereas *tilB* and other mutants were normal in both regards, indicating that downstream neuronal functions, such as axonal projection or synaptic neurotransmission, are affected (Kernan *et al*., 1994).

The mutations proved to be debilitating to adults, producing severely uncoordinated behaviour, but were not completely adult-lethal. Consequently, the adult phenotypes were used as selection criteria for further screens that produced a series of *no mechanoreceptor potential* (*nomp*) mutants, *nompA-D*,

and a *reduced mechanoreceptor potential* (*remp*) mutation (Kernan *et al.*, 1994). A more detailed characterization of *nompC* showed that it is expressed in both touch receptors and chordotonal organs, indicating for the first time that chordotonal and touch receptor neurons may use the same transduction mechanisms (Walker *et al.*, 2000).

While the foregoing screens were designed to detect mutations in the touch-sensitive sense organs, screening for auditory mutants was expected to reveal defective mechanoreception in the chordotonal organs. The ch of Johnston's organ have long been recognized as a site of auditory sensitivity in insects (Yack, 2004). A screen for auditory mutants was devised based upon an inability of males to initiate mating behaviour (in fact, male–male courting) when exposed to an amplified courtship song played through a loudspeaker (Eberl *et al.*, 1997). Subsequently, an electrophysiological approach established that the Johnston's organ in *Drosophila* was the auditory organ. First, mutants in *atonal* (*ato*), that lack ch organs altogether, were shown to be behaviourally deaf and lacking extracellular potentials in the antennal nerve (Eberl *et al.*, 2000). Next, investigation of the touch- and sound-insensitive mutants available at the time indicated that both modalities were affected in mutants; however, a few hearing-specific mutations were isolated (Eberl *et al.*, 2000), pointing to possible chordotonal-specific defects.

### 3.2 MUTATIONS AFFECTING CH AND ES ORGANS

Upon isolation of mutations affecting chordotonal organ and external mechanoreceptor functions, genetic and cellular analyses were carried out to narrow down the location of the defect and determine the function of the affected gene. Mutations arising from these and similar screens can be divided into three categories: (1) those affecting putative or proven ion channels, (2) those affecting structural integrity of the organule, and (3) those affecting intraflagellar transport. As will be seen below, the categories are not necessarily clear-cut but help in apportioning gene function.

#### 3.2.1 *Mutations affecting the ion channels*

The *transient receptor potential* family of cation channel genes was first detected in *Drosophila* due to the effect of mutations upon the electroretinogram response in the eye. These genes are phylogenetically conserved in eukaryotes and have been implicated in sensing a variety of modalities, including vision, mechanoreception, thermosensation and olfaction (Liedtke and Kim, 2005; Montell, 2005). In all, *Drosophila* has 13 TRP family members (Montell, 2005), of which 10 have been characterized to a greater or lesser extent (Table 1). Three TRP genes – Trp, Trpl and Trpgamma – are associated with visual transduction. Two closely related genes – *inactive* (*iav*) and *Nanchung* (*Nan*) – are associated with mechanotransduction in

TABLE 1 The transient receptor potential family of genes in *Drosophila*

| Function | Location | Gene | Gene Family |
|---|---|---|---|
| photo-reception | eyes | TRP | **TRPC** |
| | | TRPgamma | |
| | | TRPL | |
| | | TRPM | **TRPM** |
| mechano-reception | touch hairs | NOMPC | **TRPN** |
| | chordotonal neurons | NAN | **TRPV** |
| | | IAV | |
| noci-, thermo-reception | many sensory neurons | pyrexia | |
| | | dTRPA3 | |
| | CNS neurons, neuro-endocrine cells + others | dTRPA1 | **TRPA** |
| | gustatory, md neurons | Painless | |
| sperm orientation | sperm | AMO | **TRPP** |
| | | dTRPML | **TRPML** |

chordotonal organs. One – the *no mechanoreceptor potential C* (*nompC*) gene – is associated with mechanoreception in touch receptors. Others, discussed later, are associated with nociception and thermosensation.

*nompC*, the first TRP family member to be directly associated with insect mechanotransduction (Walker *et al.*, 2000), was isolated in a screen for touch insensitivity. Mutation of this gene results in a loss of mechanoreceptor potential in the tactile hairs and an approximately 50% reduction in auditory response (Kernan *et al.*, 1994; Eberl *et al.*, 2000; Walker *et al.*, 2000). The gene is expressed in both external and chordotonal sense organs (Walker *et al.*, 2000). Sequencing revealed that the gene encodes a protein with six transmembrane domains and 29 internal ankyrin repeats (Walker *et al.*, 2000). A theoretical study indicates that the 29 repeats constitute a single complete turn of a helix, perhaps providing the mechanical and structural properties for binding the channel components to the microtubules of the tubular body (Howard and Bechstedt, 2004). In fact, the large protein complex associated with the ankyrin repeats could constitute the microtubule-integrated cones linking the membrane and tubular body, visible under the transmission electron microscope and postulated as the site of stimulus transduction (Thurm *et al.*, 1983; Toh, 1985; Keil, 1997a).

The *nompC* protein has only a small extracellular domain and it is possible that it dimerizes with another protein, providing an extracellular anchor to the channel (Chung *et al.*, 2001). A prime candidate for dimerization is the *nompA* gene product, a transmembrane protein with a zona pellucida (ZP) domain and extensive plasminogen N-terminal (PAN) modules that characteristically bind with the extracellular matrix (Chung *et al.*, 2001). The *nompA* mutation substantially reduces both touch and sound sensitivity (Kernan *et al.*, 1994; Eberl *et al.*, 2000), and shows a detachment of the dendrite tip from the base of the external sense organ in external mechanoreceptors and

detachment of the dendrite tip from its termination in the cap in chordotonal organs (Chung *et al*., 2001). The protein is expressed in the expected cells – the sheath cells of es and ch organs – and is located extracellularly in the dendritic sheath (es) and cap (ch). While heterodimerization of *nompA* and *nompC* has not been proven, it is suggested by the complementary expression of *nompC* in the neuron and *nompA* in the sheath cell (Chung *et al*., 2001; Jarman, 2002). The predicted location of *nompC* within dendrites at the dendritic sheath/dendrite junction and cap/dendrite junction has not yet been confirmed through expression studies.

The failure of *nompC* mutation to completely eliminate sound-evoked potentials in the Johnston's organ suggests that an additional ion channel is involved in chordotonal organ transduction. Two *Drosophila* ion channels of the TRP vanilloid (TRPV) family were isolated by homology to *C. elegans* channel genes, *osm-9* and *ocr-2*, involved in mechanotransduction (Kim *et al*., 2003). Named *Nanchung* and *inactive*, both are expressed only in chordotonal organs and are localized in the cilium below the ciliary dilation (Kim *et al*., 2003; Gong *et al*., 2004). The two proteins are interdependent, suggesting that they conjoin to form an active complex (Gong *et al*., 2004). Interestingly, this localization places the chordotonal-specific channels at a location where there is no obvious direct anchorage to extracellular components, unlike the putative *nompA*/*nompC* interaction that takes place at the distal dendrite (Fig. 2).

### 3.2.2 *Mutations affecting organule integrity*

The component cells of mechanoreceptive sense organs display well-developed junctional regions, especially between the dendrite and the enwrapping scolopale or thecogen cell (Wolfrum, 1990, 1991a, 1997; Yack and Roots, 1992). Mutations that disrupt cell–cell junctions frequently disrupt mechanoreception, especially in chordotonal organs where they are highly developed and strong mechanical coupling between dendrite and scolopale appears to be crucial.

Mutants in the *Drosophila* ortholog of a gene encoding an EB1 protein show a substantially reduced sound-evoked potential (Elliott *et al*., 2005). EB1 proteins are microtubule plus end tracking proteins primarily associated with microtubule termination sites or at associations between the microtubule cytoskeleton with the cell cortex. In mutants, Johnston's organ and other chordotonal organs have normal cell ultrastructure but show a general disorganization of chordotonal units; for example, the dendrites of grouped sense organs are not properly aligned. EB1 is expressed in the scolopale cell, both in the scolopale region and around the inner dendritic segment, in the distal attachment region of the cap cell and in the ligament cell (Elliott *et al*., 2005). Both regions have concentrations of microtubule-backed hemiadherens junctions (Yack, 2004). The EB1 protein interacts with *Drosophila* short stop protein, a member of the

spectraplakin family that is the third largest protein in the *Drosophila* genome (Roper *et al*., 2002). The protein, whose main role appears to be linking actin and microtubules (Gregory and Brown, 1998), is also associated with the well-developed hemiadherens junctions at muscle to tendon attachments (Prokop *et al*., 1998). In chordotonal organs, the *short stop* mutation results in general disorganization, similar to EB1 mutants, as well as failure of dendritic tip anchorage in mononematic cilia and microtubule defects within the dendrites. Both EB1 and short stop could play a structural role in maintaining the integrity of hemidherens junctions.

Crumbs, a protein associated with zonula adherens junctions, and neurexin, a membrane-spanning protein associated with pleated septate junctions, both show very strong expression in chordotonal organs (Tepass *et al*., 1990; Baumgartner *et al*., 1996), indicating that junctional proteins are particularly enriched in these sense organs. Neurexin is most strongly expressed at the septate junctions between the cap and scolopale cells (Baumgartner *et al*., 1996). A mutation results in splaying of the scolopale, suggesting that longitudinal tension of the chordotonal organs has been disrupted. This phenotype is also seen in mutations for the *Drosophila MyosinVIIA* gene (also known as *crinkled*) that experience apical detachment due to morphological defects in the cap. *MyoVIIa*, a non-muscle myosin, is expressed in both the scolopale cell and neuron; in the scolopale cell, expression is strongest along the scolopale rods and at scolopale-cap junctions. In neurons, it is expressed at the cilium base where dendrites bind to the scolopale cell via hemiadherens junctions (Todi *et al*., 2005). *MyoVIIa* mutations result in complete loss of sound-evoked potentials (Todi *et al*., 2005) and reduced TEPs and MEPs in tactile receptors (Todi *et al*., 2004).

In summary, junctional complexes are well developed in chordotonal organs where they appear to be important for normal functioning. The same junctional complexes are present in external mechanoreceptors as well as other modalities; however, they are less developed and although specific tests have not always been carried out, they appear to play less of a role in sensory transduction.

### 3.2.3 *Mutations affecting intraflagellar transport*

Type I sensory neurons have in common a ciliary structure in the dendrite. In chordotonal organs the cilium itself appears to be involved in mechanotransduction, while in external mechanoreceptors the tubular body, distal to the cilium, plays this role. In either case, transduction-associated proteins must be transported into or beyond the cilium. Defects in intraflagellar transport would be expected to affect all type I sense organs – gustatory, olfactory, touch receptors and chordotonal.

The kinesin II-mediated intraflagellar transport (IFT) system is common to eukaryotes and is based on a three-component system: two motor subunits

and a kinesin–associated protein. In *Drosophila, Klp64D*, *Klp68D* and *DmKap* are the motor subunits and associated protein, respectively (Pesavento *et al*., 1994; Ray *et al*., 1999; Sarpal and Ray, 2002). Axonemal assembly in chordotonal organs is disrupted in the mutations *Klp64D* and *DmKap* and the auditory response is reduced or eliminated (Sarpal *et al*., 2003). In *DmKap* the cilia of Johnston's organ ch are missing altogether and in *Klp64D* their integrity is affected and the ciliary dilation is extended distally (Sarpal *et al*., 2003). The effect on other sensillum types was not investigated.

Avidor-Reiss *et al*. (2004) used a comparative genomics approach to isolate *Drosophila* genes associated with compartmentalized cilia, the type found in sensilla. The screen revealed genes already known to be involved in IFT as well as a number of new candidates. An accompanying screen for mechanoinsensitive flies, similar to those detailed above, revealed that mutations in a set of these IFT candidates result in loss or reduction of outer dendritic segments in both mechanoreceptors and chemoreceptors. *Regulatory factor X* is another transcription factor involved in intraflagellar transport (Dubruille *et al*., 2002). *Rfx* mutations in *Drosophila* cause defective cilium formation and show abnormal mechanosensory, gustatory and olfactory function. Dendrites of the ciliated neurons are structurally abnormal and the ciliary rootlets are missing from chordotonal organs. The *nompB* gene, isolated from a screen for uncoordinated adults (Kernan *et al*., 1994), encodes an IFT protein homologous to the IFT88/Polaris/OSM-5 family, a component of IFT particles, and is localized to sensory cilia (Han *et al*., 2003). The mutation causes a loss of transduction in both chordotonal organs and external mechanoreceptors (Eberl *et al*., 2000). As expected for IFT genes, mutations affect a diversity of ciliated receptors and *nompB* mutants show defective chordotonal, campaniform, and olfactory cilia (Han *et al*., 2003).

Another gene involved in ciliary function is *uncoordinated* (*unc*), encoding a novel protein whose precise function remains unknown (Baker *et al*., 2004). It is associated with the basal bodies immediately proximal to the ciliary region. The protein has a core ciliogenic function because mutants affect sperm motility and all ciliated neurons. In *unc* mutants, the sensory cilia are absent or deformed in chordotonal, external mechanosensory and olfactory sensilla (Baker *et al*., 2004). The *Drosophila* pericentrin-targeting (PACT) protein is also associated with basal bodies. The mutant affects ciliated neurons and shows characteristic uncoordinated adult phenotypes (Martinez-Campos *et al*., 2004). The cilia of both olfactory and chordotonal sensilla show a disruption similar to that seen in *unc* mutants.

### 3.2.4 *Activation of chordotonal mechanoreceptors*

The precise mechanism by which ion channels of chordotonal organs are activated remains unknown (reviewed by French, 1988; Field and Matheson, 1998). The evidence above suggests that ch organs could possess two types of

ion channels, only one of which, the putative Nan/iav dimer channel, is absolutely required for sensitivity of the *Drosophila* Johnston's organ. The channel is located in the ciliary region. Many authors have suggested that ch are activated by stretching of the cilium and its axoneme along the longitudinal plane, some say that the axoneme bends, applying differential stretch to the membrane. At the ultrastructural level, some insect ch show such ciliary bending just distal to the basal bodies (Fig. 2) but this has not been linked directly to stimulation. Also, in some insect chordotonal organs, electron microscopy reveals extracellular structures traversing the inner sensillum lymph space, connecting the cilium and the scolopale cell (Yack and Roots, 1992) and potentially anchoring the ion channels. It would not be surprising if secretions from the scolopale cell into the lymph cavity surrounding the dendrites were also important for transduction.

The possibility of ciliary activity playing a role in sensory transduction has been resurrected (see review by French, 1988) since the discovery that mosquito antennae show active vibrations, detected using sensitive laser vibrometry (Gopfert and Robert, 2000, 2001). Such non-muscle activity of the receptors has proved to be widespread among the Diptera and is also found in vertebrate auditory hair cells where it acts to enhance sensitivity and tuning of the receptors (Gopfert and Robert, 2000; Robert and Gopfert, 2002). Examining the available hearing-defective mutations in *Drosophila*, Gopfert and co-workers confirmed that live adults exhibit spontaneous antennal oscillations and showed that hearing-defective mutants have reduced or eliminated antennal motor response that largely matched their electrophysiologically tested auditory response (Gopfert and Robert, 2003; Gopfert *et al*., 2005). Insect orthologs of *prestin*, that encodes the motor protein of mammalian hair cells, have been identified and shown by *in situ* hybridization to be expressed in Johnston's organ of *Drosophila* and mosquitoes (Weber *et al*., 2003) but unfortunately detailed expression patterns, and mutants, are not yet available.

Two chordotonal organ-specific mutations, *touch-insensitive larva B* (*tilB*) and *beethoven* (*btv*), isolated from behavioural screens are considered as candidates for involvement in active dynamics of the cilium (Eberl *et al*., 2000). Mutations in *btv* result in expansion of the ciliary dilation as well as loss of the paracrystalline inclusion in Johnston's organ chordotonal neurons (Eberl *et al*., 2000) as well as loss of the outer ciliary dilation in lateral chordotonal organs of late embryos (Caldwell *et al*., 2003). The precise identity of *btv* has not been determined but has been narrowed down to two feasible candidates: a dynein heavy chain gene that could be involved in intraflagellar transport, or a cadherin gene that could be involved in maintaining cell–cell contacts (Caldwell and Eberl, 2002; Todi *et al*., 2004). *tilB* is also implicated in the function of the cilium because it causes male sterility and defective sperm tail axonemes but the mutant shows no ultrastructural defects in chordotonal organs (Eberl *et al*., 2000). Todi *et al*.

(2004) speculate that active movement of the cilium through contractile twisting keeps it under tension and that channels are located at the base of the cilium where they are activated by changes in the ciliary angle. Another possibility to be considered is Wolfrum's theory (1991b) that the ciliary rootlets are the source of active movement, reacting to changes in tension along the longitudinal axis of the dendrite. Tensioning the ciliary membrane could change the sensitivity of the ion channels. Ciliary rootlets are immunoreactive to α-actinin and centrin (Wolfrum, 1991b, 1992, 1997), shown to be involved in contraction of algal flagellar rootlets. This hypothesis separates the active movement (ciliary rootlets) from the ion channels themselves located in the cilium.

The various hypotheses about chordotonal organ force transmission, sensory transduction and active movements remain to be tested using protein expression studies at high levels of resolution. Methods such as freeze-substitution fixation and transmission electron microscopy coupled with immunostaining techniques should prove to be informative, for example, as used to localize odorant receptor molecule epitopes and odorant binding proteins within the olfactory sensilla of *Drosophila* (Shanbhag *et al.*, 2005; Benton *et al.*, 2006).

## 4 Multidendritic sensory neurons

Multiple dendrite (md) or multiterminal neurons are generally proprioceptive in function (Wright, 1976). They are commonly found at leg and wing joints where they respond electrophysiologically to extension of the appendages. In soft-bodied caterpillars, they have also been shown to fire in response to the application of pressure to the body wall (Grueber *et al.*, 2001) and in adult tsetse flies to abdominal distension (Anderson and Finlayson, 1978). A single sense organ called the dorsal longitudinal stretch receptor, found in the thoracic and abdominal segments, is highly conserved in insects (Finlayson, 1968). Typically, its dendrites span the length of the segment and are associated with a lineage-related support cell or a muscle fibre. The *Drosophila* homolog, called the dorsal bipolar dendrite sense organ, has a simple lineage, arising as the result of a single division of a precursor cell (Brewster and Bodmer, 1995), its sister cell becoming a support cell with a number of glia-like properties (Halter *et al.*, 1995). Along with an adjacent md neuron it is the only sense organ outside of the antenna that utilizes *amos* as the proneural gene (Goulding *et al.*, 2000; Huang *et al.*, 2000) and has an unusual axonal projection in the CNS (Merritt and Whitington, 1995).

A *Drosophila* gene, named *pickpocket1* (*ppk1*) is a potential ion-channel gene in mechanoreceptive multidendritic neurons. Its expression is restricted to a specific subset of md neurons with widely ramifying subhypodermal

dendrites located in each segment of the embryo and larva (Adams *et al.*, 1998). The PPK protein is a member of the degenerin/epithelial sodium channel (DEG/ENaC) gene family. It was detected in *Drosophila* by orthology to *C. elegans'* DEG/ENaC genes, which are known to play a mechanoreceptive role (Goodman and Schwarz, 2003). Further support for a proprioceptive role for this subset of md neurons comes from observations of crawling defects in mutant larvae (Ainsley *et al.*, 2003).

Some more unusual functions of insect md neurons have been determined. Electrophysiological investigations indicate a thermoreceptive function for multiterminal sensory neurons in the wings of butterflies, perhaps related to regulation of basking behaviour (Schmitz and Wasserthal, 1993). A search for temperature-sensitive neurons in *Drosophila* by Liu *et al.* (2003b) using $Ca^{2+}$-sensitive fluorescence resonance energy transfer demonstrated that some identified multiple-dendrite neurons increased their activity during heating and reduced it on cooling. However, a temperature response of one kind or another was found in many different classes of neurons, raising questions about the nature and specificity of any temperature-sensitive ion channels.

A screen for *Drosophila* mutations affecting response to noxious stimuli (nociception) led to the discovery of the gene *painless* that is expressed in multidendritic neurons (Tracey *et al.*, 2003). Mutations in *painless* abolish the normal vigorous escape response to noxious heat and strong mechanical stimuli. *Painless* encodes a member of the TRPN ion channel family most closely related to *Drosophila nompC*. Its closest mammalian relative is TRPA1/ANKTM1, an isothiocyanate (wasabi) receptor that detects spicy, pungent sensation. Interestingly, *painless* is also expressed in gustatory receptors on the labial palps, tarsi and wing margin where it mediates an avoidance response to isothiocyanates in food (Al-Anzi *et al.*, 2006), indicating that the molecular mechanisms for detecting plant–derived toxins are conserved between insects and mammals, and perhaps indicating an evolutionary link between multidendritic neurons and gustatory neurons (see Section 8.3).

*Pyrexia* (also called dTRPA2), related to *painless*, is another member of the TRPA family involved in thermosensation. It is expressed widely in the neuropil of the CNS and in sensory and central neurons (Lee *et al.*, 2005). Its expression in sensilla seems to be relatively non-specific, being found in md neurons as well as other external sense organs. Mutants show different temperature preferences to wild types and a susceptibility to paralysis at high temperatures. It is likely that ion channel protein encoded by *pyrexia* prevents neurons from firing inappropriately under high-temperature stress (Lee *et al.*, 2005). A third member of the four-part *Drosophila* TRPA family, dTRPA1, is the only member of the family involved in larval thermotaxis, as revealed by assaying thermal preference behaviour after deleting gene function using RNAi techniques (Rosenzweig *et al.*, 2005).

dTRPA1 is expressed in a small set of neurons in the larval brain, in neuroendocrine cells of the corpus cardiacum and two pairs of unidentified, anteriorly located cells adjacent to the larval mouth hooks. Unfortunately, the authors did not determine whether the latter cells are neurons, making it difficult to determine whether the gene is required in the peripheral nervous system (Rosenzweig *et al.*, 2005). It is not expressed in multidendritic neurons or chordotonal neurons. Reciprocal testing of *painless* mutants and dTRPA1-compromised larvae in thermotaxis and noxious stimulus avoidance assays indicate that the two classes of behaviours are mediated by distinctly different ion channel proteins (Rosenzweig *et al.*, 2005).

The widespread expression of *painless* in md neurons, combined with the foregoing results, suggests that many of these sensory neurons may be multimodal, given their responses to mechanical stimuli, noxious thermal stimuli and benign temperature shifts. Taking axonal projections as a guide, the md neurons can also be divided into subgroups according to the nature of their axonal projections in the CNS that correlates, to some extent, with the identity of the proneural gene specifying the type of lineage from which they are derived (Merritt and Whitington, 1995; Schrader and Merritt, 2000; Grueber *et al.*, 2001). Evidently, md neurons constitute a diverse class of receptor modalities and, unlike the sensory neurons associated with organules, ultrastructural features do not provide many clues as to their function.

Another unusual sensory neuron that technically belongs to the multidendritic class is the single-neuron photoreceptor associated with the genitalia of *Papilio* butterflies that increases its firing rate on exposure to light (Arikawa *et al.*, 1980). Structurally, they do not resemble the retinula cells constituting the photoreceptors of the eye, rather the neuron gives off a number of processes that subdivide into tubular processes, all tightly packed against the neuronal soma (Miyako *et al.*, 1993). It would be most interesting to know whether these extraocular photoreceptors express genes typical of the eyes or of the discrete non-photoreceptive sensory organules, providing an insight into whether photoreception in this sensory neuron has evolved independently of ocular receptors, or through co-option of photoreceptor developmental genes, or is due to ectopic expression of the eye developmental network.

## 5 Transduction in olfactory and gustatory receptors

### 5.1 OLFACTORY RECEPTORS

Olfactory sensilla are usually innervated by several neurons (Zacharuk, 1980). A characteristic of their structure is the high prevalence of dendritic

branching distal to the cilium, presumably increasing the available membrane surface area for location of the receptor molecules (Zacharuk, 1980; Keil and Steinbrecht, 1984). The cuticle of the sensory hair or peg surrounding the dendrites is fenestrated with fine pores allowing volatile molecules to access the fluid surrounding the dendrites. This fluid originates in the outer receptor lymph cavity and is secreted by the tormogen and trichogen cells. A thecogen cell is associated with olfactory sensilla, however, the dendritic sheath that is secreted by the thecogen cell in other sensillum types is often missing or reduced (Kuhbandner, 1985). Insect olfactory receptors are concentrated on the antenna but are also found in other locations, for example, in Diptera they are found on the maxillary palps and, in some cases, on the ovipositor (Zacharuk, 1980).

Insect olfactory neurons respond to a wide variety of volatile chemicals as demonstrated by "single unit" electrophysiological recordings, in which an electrode inserted at the base of a single olfactory sense organ reveals the response characteristics of the individual neurons. Electrophysiological and behavioural assessments have shown that olfactory sensilla respond to stimulants that are linked to ecological or physiological requirements of the species under examination. Many insects utilize volatile pheromones for detection of mates and it is not surprising that their olfactory receptors are finely tuned to these chemicals (Blomquist and Vogt, 2003).

The first step in odour reception is passage of the volatile molecules through cuticular pores into the receptor lymph. Here, odorants can bind to odorant binding proteins (OBPs) that may enhance the second step, presentation of the ligand to the odorant receptor molecules embedded in the dendrite membrane. These are known as perireceptor events. The third step is activation of a signalling cascade resulting in the generation of receptor potentials.

### 5.1.1 *Odorant binding proteins*

Odorant binding proteins (OBPs) are located in the receptor lymph space where they are secreted by the trichogen or tormogen cells, as shown by immunocytochemistry at the transmission electron microscope level (Steinbrecht *et al.*, 1992; Shanbhag *et al.*, 2005). OBPs were first isolated from silkmoth where they bind pheromone molecules (Vogt and Riddiford, 1981), earning the name pheromone-binding proteins. Subsequent experiments on moths showed that "generalist" OBPs, for example, those associated with sensitivity to plant volatiles, are expressed in many olfactory sensilla while specialist pheromone-binding proteins are expressed in the male sensilla only (Blomquist and Vogt, 2003).

Complete genome screening in *Drosophila* revealed that the OBP family comprises 51 potential genes (Hekmat-Scafe *et al.*, 2002). They are identified as paralogs by the conservation of spaced cystines and intron

insertion sites. Otherwise, they show low sequence similarity but their tendency to be located in clusters in the genome suggests they have arisen by duplications followed by rapid divergence. Of the 51 genes, several have been investigated with regard to site of expression and function (summarized in Hekmat-Scafe *et al*., 2002).

Several functions have been ascribed to OBPs, the simplest being binding and transport of hydrophobic odorants through the receptor lymph to the dendritic membrane. More recent studies indicate that they may have a role in defining the specificity of olfactory and gustatory neurons. The *lush* mutant of *Drosophila*, defective in the gene *obp76a*, shows reduced sensitivity to ethanol (Kim *et al*., 1998) and complete insensitivity to a volatile pheromone (Xu *et al*., 2005), mediated through antennal olfactory receptors. Further, in the *lush* mutant, the background firing rate of the pheromone-sensitive neurons is reduced, indicating that this OBP may interact directly with odorant receptor proteins in the dendritic membranes (Xu, 2005; Xu *et al*., 2005). The detailed mode of action of OBPs remains to be elucidated, although at present it appears that they act to enhance or block the access of odorants to the receptor membrane. Their diversity, combined with the diversity of the odorant receptors, helps explain the specificity and sensitivity of olfactory neurons.

Investigations of Lepidoptera have revealed two gene families that could encode proteins involved in or associated with olfactory transduction; the sensory neuron membrane proteins (SNMPs), members of the CD36 family of membrane-bound proteins, are expressed in olfactory dendrites (Rogers *et al*., 2001), and the guanylyl cyclase MSGC-I is expressed in the cell body and dendrites of olfactory neurons as well as in the brain (Nighorn *et al*., 2001). Mutant analyses or gene knockdowns are needed to analyse their function further.

Surprisingly, many OBPs are expressed in gustatory receptors (Galindo and Smith, 2001) where their role has not been examined in detail. Targeted death of the *obp*-expressing support cells in *Drosophila* gustatory sensilla caused loss of gustatory sensitivity; however, this may be due to effects on other functions of the support cells rather than their production of OBP (Galindo and Smith, 2001).

### 5.1.2 *Odorant receptor molecules*

Our knowledge of odorant receptor molecules expanded greatly when a large number of *Drosophila* genes encoding odorant receptors was identified using a bioinformatics screen, based upon a search for genes encoding proteins with seven membrane-spanning regions originally identified as characteristic of odorant receptors in mammals and *C. elegans* (Clyne *et al*., 1999; Gao and Chess, 1999; Vosshall *et al*., 1999; recent reviews by Vosshall, 2003; de Bruyne and Warr, 2006; Hallem *et al*., 2006). With the

completion of the *Drosophila* genome project, the number of *odorant receptor* (*Or*) genes increased to 60, encoding 62 proteins (Robertson *et al*., 2003). Outside of the membrane-spanning region, predicted *Or* proteins are highly variable; however, analysis of intron evolution indicates that they have a common ancestor that is shared with gustatory receptor proteins (see Section 5.2) (Robertson *et al*., 2003). The diversity of *Or* genes immediately posed questions about the specificity of genes to individual olfactory neurons and, more broadly, about whether other insects show the same degree of diversity in a family of receptor molecules that would be expected to come under concerted evolutionary pressure in association with ecological specialization and detection of pheromones.

To answer the first question, individual *Or* genes were mapped to their neurons using gene-specific promoters to drive green fluorescent protein (GFP) in the neurons and by driving cell death genes followed by electrophysiological confirmation of insensitivity. The approach revealed that a single *Or* gene can render an olfactory neuron sensitive to a broad range of odorant chemicals (Dobritsa *et al*., 2003) and different receptors have different breadth of response, some being specific to a small range of volatiles, while others are broadly tuned (Hallem *et al*., 2004). The identity of the *Or* gene dictates the characteristics of the neuron such as the response specificity and resting firing rate of the neuron, as shown by ectopically expressing the genes in receptors whose native *Or* gene expression was genetically deleted, called the ''empty neuron'' approach (de Bruyne and Warr, 2006). These approaches have led to the conclusion that a majority of olfactory receptor neurons express a single *Or* gene (Hallem *et al*., 2004); however, some neurons routinely express combinations of genes (Goldman *et al*., 2005). An example of fine-tuning is the exquisite sensitivity of male silkmoth olfactory sensilla to the female pheromone (Kaissling, 1996). Male-specific *Or* genes were isolated from *Bombyx mori*, one of which is tuned to bombykol, the sex pheromone, and the other to bombykal, the oxidized form of bombykol. The bombykol and bombykal *Or* genes are each expressed in one neuron of a two-neuron olfactory sensillum on the male antenna, perhaps facilitating the sensitive detection of pheromone blend ratios (Nakagawa *et al*., 2005).

Comparison of *Or* diversity in *Drosophila* with *Anopheles gambiae* and *Heliothis virescens* revealed that *A. gambiae* has a similar diverse set of 79 *Or* genes (Hill *et al*., 2002) and there are at least 60 in the honey bee *Apis mellifera* (Robertson, cited in Jacquin-Joly and Merlin, 2004). *Drosophila* and *Anopheles* each possess uniquely expanded subfamilies of *Or* genes, perhaps providing olfactory sensitivity for detection of species-specific ecologically relevant volatiles such as fruit odours in *Drosophila* and vertebrate host odours in the mosquito (Hill *et al*., 2002). In a revealing experiment, two sex-specific female *Anopheles Or* genes, selected as prime candidates for encoding vertebrate host-specific receptors, were expressed

in *Drosophila* using the "empty neuron" approach. The receptors proved to respond electrophysiologically to sweat-related odorants, a response not present in unmodified *Drosophila* (Hallem *et al*., 2004). Thus, the identity of the *Or* gene alone was sufficient to elicit a gene-specific neural response in a distantly related species, indicating that the cellular machinery associated with signal transduction must be highly conserved.

Further evidence for the conservation of the basic transduction pathway comes from the across-species conservation of a particular *Or* gene, *Or83b*. A recognizable ortholog is conserved in many insects examined, including Diptera, Lepidoptera, Coleoptera and Hymenoptera ( Krieger *et al*., 1996; Hill *et al*., 2002; Melo *et al*., 2004; Pitts *et al*., 2004; Jones *et al*., 2005). In *Drosophila,* it is expressed in almost all olfactory neurons although it does not appear to act as a functional, stand-alone receptor. Rather, loss of function indicates that it is essential for normal olfactory physiology and behaviour, and protein localization studies indicate that it is essential for transport of the co-expressed Or proteins from the cell body into the dendrite (Larsson *et al*., 2004). Its role might be to heterodimerize with Or proteins, traffic them through the cilium and help them insert in the membrane. This conserved, essential function in Or protein trafficking has prevented *Or83b* from diverging across species while allowing the functional divergence of its Or partners.

Olfactory transduction is thought to utilize a G protein-mediated signal transduction cascade, based upon the similarity of Or proteins to a family of receptors called membrane-bound G protein-coupled receptors (GPCRs). Recently, Benton *et al.* (2006) re-examined protein structure and established that *Drosophila* Or proteins are not as closely related to vertebrate and nematode odorant receptors as first thought, as they possess a novel membrane topology. This result brought into question the untested assumption that Or proteins interact with G protein-coupled transduction mechanisms to activate receptor potentials, although there is some evidence that this pathway is involved (see Benton *et al*., 2006). Future research will undoubtedly focus on this particular pathway and its role in insect olfaction.

A fundamental issue that has yet to be addressed in any detail is what genetic mechanisms determine the identity of the *Or* expressed in each sensillum? One possibility is that transcription factors of the POU domain family could be involved. The *abnormal chemosensory jump 6* (*acj6*) gene was isolated from a behavioural screen for absence of the normal jump escape reflex on exposure to concentrated chemical vapours (McKenna *et al*., 1989). *Acj6* proved to be a mutation in a POU domain gene, a member of a family of transcription factors known to be involved in nervous system development. *Acj6* is expressed in a subset of olfactory neurons and the mutant produces abnormal odour specificity perhaps through a role in regulating receptor gene expression in a subset of olfactory sensilla (Clyne *et al*., 1999). It is possible that additional, interacting

transcriptional factors will be found that combine to determine the complex pattern of *Or* expression in olfactory sensilla (Clyne *et al*., 1999; Hallem and Carlson, 2004).

The axons of olfactory sensilla project into clustered olfactory glomeruli in the antennal lobes. There are approximately 50 glomeruli in each lobe, individually recognizable by their position (Laissue *et al*., 1999). The projections of olfactory sensilla have been studied in detail, with the *Drosophila* olfactory system emerging as an informative model for olfactory specificity and interactions of the primary sensory axons with olfactory interneurons in the glomeruli (Fishilevich and Vosshall, 2005, reviewed by Jefferis and Hummel, 2006).

### 5.2 GUSTATORY RECEPTORS

Gustatory sensilla are generally multi-innervated with several gustatory neurons and, frequently, a mechanoreceptive neuron whose dendrite terminates at the base of the hair. The gustatory neurons have single, unbranched dendrites that travel within a canal in the cuticular hair or peg to a pore at the tip. The dendrites broaden beyond the cilium, but otherwise show no distinguishing features at the ultrastructural level that could be associated with transduction. The mechanoreceptive neuron possesses a tubular body similar to that seen in purely mechanoreceptive sense organs. Thus, most gustatory sensilla are in fact multimodal, responding to both touch and taste. In flies, most gustatory sensilla have four gustatory neurons plus the mechanoreceptor (Stocker, 1994).

Gustatory responses in insects have long been the subject of electrophysiological investigation and their sensitivity to salts and other gustatory stimulants has been extensively tested (reviewed by Dethier, 1976; Stocker, 1994; Rogers and Newland, 2003). The first gustatory receptor (Gr) proteins in insects were identified using the same bioinformatics approach that revealed the *Or* genes (Clyne *et al*., 2000). The *Gr* genes share the seven membrane-spanning region originally identified as characteristic of olfactory receptors. In *Drosophila*, 68 receptor molecules have been identified, encoded by 60 genes, some showing alternative splicing (Robertson *et al*., 2003; reviewed by Amrein and Thorne, 2005; Hallem *et al*., 2006). In the mosquito *Anopheles gambiae*, 76 gustatory receptor genes were identified (Hill *et al*., 2002). Like the Or family, the Gr family is highly divergent, showing sequence identity of between 7% and 50% (Scott *et al*., 2001). All of the *Gr* genes share a common motif in the carboxyl terminus. The same motif is found in some, but not all, *Or* genes, indicating a common evolutionary origin of the two families. A comprehensive phylogenetic analysis of Or and Gr families in *Drosophila* indicated that the Ors are a subset of the Gr family (Robertson *et al*., 2003). Interestingly, Or83b, the widespread, phylogenetically conserved olfactory receptor is closest in sequence

to gustatory receptor genes, reinforcing its position as a unique receptor type (Robertson *et al*., 2003). A small number of Gr family members are expressed in the antenna and/or maxillary palp of *Drosophila* (Dunipace *et al*., 2001; Scott *et al*., 2001), where they are presumably acting as olfactory receptors because comprehensive ultrastructural studies of the antennal sensilla have failed to reveal any with the morphological characteristics of gustatory sensilla (Shanbhag *et al*., 1999), indicating the Grs are perhaps not exclusively gustatory. Similarly, a small number of Ors are expressed in gustatory receptors (Scott *et al*., 2001). Multiple *Gr* genes can be expressed in one neuron (Thorne *et al*., 2004; Wang *et al*., 2004).

Some gustatory neurons possess ion channels belonging to the DEG/ENaC gene family. The first member of the group to be well characterized, *pickpocket1* (*ppk1*), is mentioned above in relation to its expression in a subset of multidendritic neurons (Liu *et al*., 2003a). There are 25 DEG/ENaC genes present in the *Drosophila* genome, several of which are expressed in the gustatory system (Galindo and Smith, 2001). The genes *ppk11* and *ppk19* are expressed in both larval and adult gustatory neurons, including the labellar, tarsal and wing gustatory sensilla. Expression of a dominant negative construct or RNAi resulted in behavioural defects associated with a deficiency in detecting low salt concentrations, indicating these genes are involved in detecting $Na^+$ and $K^+$ salts (Liu *et al*., 2003a). Another family member, *ppk25,* is expressed in the legs and antennae of males and is required for male response to female pheromones (Lin *et al*., 2005). *Gr68a* has also been implicated in pheromone detection. It is male-specific, and is expressed in the neurons of the male-specific gustatory sensilla on the forelegs (Bray and Amrein, 2003). Inactivation of the *Gr68a*-expressing neurons using targeted cell-death constructs or RNAi resulted in reduced mating performance of males, likely to be associated with a failure to detect the female pheromone (Bray and Amrein, 2003). The same gustatory sensilla also express a novel male-specific gene, *CheB42a* (Xu *et al*., 2002; Lin *et al*., 2005). *CheB42a* and another male-specific gene *CheA29a* each encode a novel protein that is likely to be soluble and secreted (Xu *et al*., 2002), however, a role in pheromone reception has yet to be determined. Their expression in the support cells suggests that they could have a role analogous to OBPs but they do not share any sequence similarity.

### 5.3 HYGRO- AND THERMORECEPTORS

These sensilla tend to be found on the antennae. Hygroreceptors are often associated with peg-like sense organs on the antennae that have no pore connecting the dendrites to the outside, and thermoreceptors in pegs or hairs display spiral lamellation of the distal dendritic membrane (Altner and

Prillinger, 1980; Altner and Loftus, 1985). Both receptor types are likely to be evolutionarily derived from olfactory sensilla, based on their axonal projections (Foelix *et al.*, 1989; Nishikawa *et al.*, 1995); however, their development and transduction mechanisms remain unclear. The role of temperature and humidity perception in responding to environmental variability is discussed in an accompanying article (Chown & Terblanche, 2007).

## 6 Sensillum development

The literature on genetic and cellular interactions involved in the formation of *Drosophila* sensilla is extensive and will not be reviewed in detail here (see Hartenstein, 2005). Rather, I will reiterate some of the main messages from a landmark paper by Lai and Orgogozo (2004), who reviewed the different lineages of diverse *Drosophila* sense organs published to date and defined a canonical lineage that lies behind the diversity of *Drosophila* sense organs. They caution that the *Drosophila* picture may not apply to other insects, however, the similarities in sensillum morphology across the insects hold hope that this may be the case. The lineage is based upon a five-cell organule. Different genetic interactions and cell fate decisions are utilized to give rise to the full diversity of type I and II receptors from these five cells. The first division of the precursor produces two cells, pIIa and pIIb. The daughter cell pIIa divides once more to give an inner and outer cell, while pIIb divides to give one terminal cell and its sister, pIIa, divides to give rise to the type I (ciliated) neuron and its enwrapping sheath cell.

An indication of the flexibility of the canonical lineage is gained by comparing the chordotonal and tactile sensillum lineages, in which individual cells can be homologized based on their position in the lineage, orientation of their division plane, gene expression and mutant analysis. In ch, the pIIa progeny become the cap and attachment cells, while in es they become the socket- and shaft-secreting cells, respectively. The pIIIa cell divides to give rise to the neuron and its sheath cell, termed thecogen cell in es organs and scolopale cell in ch organs. The remaining cell can become an md neuron in some ch and es lineages, or it may become a ligament cell or glial cell.

Variations on the canonical lineage can come about through lineage-specific cell proliferation by which a pIIb-derived cell undergoes additional divisions to give multiple glial cells in the wing campaniform sensilla, or multiple type I neurons in the case of the gustatory sensilla (Fig. 3). It is interesting that the homologous cell in different sensory lineages can give rise to a glial cell, the ligament cell of a chordotonal organ or a multidendritic neuron, depending on the expression of the gene *glial cells missing* (Brewster and Bodmer, 1995; Jones *et al.*, 1995), indicating that the channelling of terminal cell fate into one or other of these morphologically and functionally different cell types can be attributed to a single genetic switch.

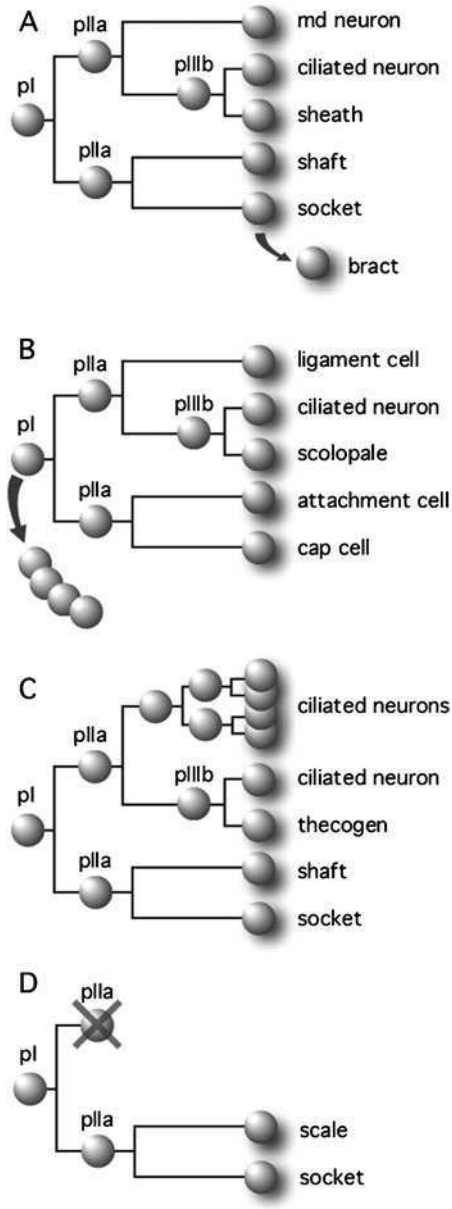



FIG. 3 Lineages of *Drosophila* sensilla. (A) A typical external sense organ lineage that gives rise to an md neuron as well as a ciliated neuron. Induction of a bract cell is indicated with an arrow. (B) A typical chordotonal organ lineage. Compare with A for homologies between the cells of the ch organ and es organ. Induction of additional ch precursors is indicated with an arrow. (C) A lineage of a multi-innervated gustatory organ. The additional neurons arise from the pIIa progeny cell. (D) The lineage of a non-innervated scale of a butterfly. Cell death is indicated by a cross. (A) to (C) adapted from Lai and Orgogozo (2004), (D) from Galant *et al.* (1998).

Lineage-specific cell death is also common, with loss of one to many cells. In adult sensilla, it is common to see one or other of the trichogen or tormogen cells degenerate after they have secreted their cuticular apparatus (Keil, 1997b). The most extreme case of cell death is seen in the development of a "solo" multidendritic neuron in the embryo, derived from an es-type lineage in which all cells, other than one of the pIIa progeny, die through apoptosis before they have undergone their terminal divisions (Orgogozo *et al*., 2002).

Another principle of sense organ development is utilization of the epidermal growth factor receptor (EGFR) pathway to recruit cells that are unrelated by lineage to take on a distinct fate in association with a sense organ. Examples include the recruitment of a bract-secreting cell in association with the leg tactile sensilla (del Alamo *et al*., 2002), and induction of oenocytes, single-celled glands underlying the insect cuticle, by the embryonic chordotonal organs (Elstob *et al*., 2001; Rusten *et al*., 2001). Recruitment is also used to produce compound sense organs composed of many replicated sensillum units. For example, the femoral chordotonal organs of *Drosophila* are established by persistent, repeated induction of new precursors by those preceding (zur Lage and Jarman, 1999).

## 7 Photoreceptors and antennal olfactory sensilla

### 7.1 THE PRONEURAL GENE, *ATONAL*, AND RECRUITMENT

Insect photoreceptors have long been recognized as evolutionarily distinct from sensilla. Superficially, the ommatidia show no morphological similarities to sensilla: the photoreceptor sensory neurons are unciliated and rhabdomeric, and the supporting cells show no homologies to sensilla, either developmental or morphological. However, developmental genetic studies show a shared requirement for the proneural gene, *atonal*, for the formation of eyes and some sensilla (Jarman *et al*., 1994). The proneural genes are instrumental in the formation of all sensilla, being required for singling out the sense organ precursor cell and dictating the sensory modality and axonal projections of the neurons (Bertrand *et al*., 2002). *Atonal* is the proneural gene required for formation of ch, whereas genes of the *achaete-scute* complex are required for formation of external sense organs such as mechanoreceptors.

Chordotonal organs differ from other sensillum types in that they frequently form in closely packed, concentrated arrays. To produce the compound organ, existing precursors repeatedly induce new precursor cells, each giving rise to a sensillum (zur Lage and Jarman, 1999). In the *Drosophila* eye, *atonal* is required for formation of photoreceptor R8 around which photoreceptors R1-7 and the support cells coalesce through

local cell inductions (Jarman *et al*., 1994; Freeman, 1997). Recruitment of cells to form ommatidia is ancient, at least present in the insect–crustacean ancestor. The ancestral crustacean, *Triops*, utilizes a recruitment mechanism to produce eight retinula cells in the same temporal pattern as insects (Melzer *et al*., 2000).

The common requirement for *atonal* in eyes and chordotonal organs poses questions about their evolutionary history. One possibility is that the *atonal*-based mechanism has been co-opted from one organ system to the other to allow recruitment of clusters by induction. In this case, the ommatidial and chordotonal organules are not homologous because they are not necessarily derived from the same type of ancestral cells. Another is that the eyes and chordotonal organs are derived from a common ancestral sense organ and are therefore homologous. At first glance, the latter seems unlikely because of the fundamentally different origin of the cells forming the organules: lineage-based *versus* recruitment. However, recent investigations of eye formation in *Drosophila* suggest a solution to the dilemma. Niwa *et al*. (2004) propose that chordotonal organs and ommatidia do indeed have a common origin, and that the different sense organs are produced by spatiotemporal expression of downstream regulatory genes. Through mutant analysis and gene misexpression in *Drosophila,* they found evidence that *eyeless*, a member of the Pax6 gene family universally associated with eye development, is in fact a segment identity-determining gene responsible for formation of the eye downstream of, or parallel to, *atonal* expression. They propose that a "protosensory" organ gave rise to both the ommatidia and the ch. If this proves to be the case, then at the cellular level the *neurons* of photoreceptors are homologous with *neurons* of chordotonal organs.

It is possible to speculate on the nature of this protosensory organ. But first, it is necessary to resolve the issue of apparent lack of homology of the non-neural cells and the different origins of these cells. If the protosensory organ comprised a single cell, albeit potentially present in large numbers, specified by *ato* expression, then different mechanisms of organule formation can come into play. To form ommatidia, the putative protosensory neuron must recruit additional cells, both neural and non-neural, to create the complete ommatidia (Fig. 4). To form chordotonal organs, the protosensory cell must undergo additional divisions to give rise to the support cells and neurons associated with ch (Fig. 4). Orthologs of *atonal*, such as genes of the *achaete–scute* complex, could then give rise to further types of lineage-based sense organs (Fig. 4). Under such a scenario, we can conclude that ommatidia and sensilla are homologous at the organule level because both are derived from ancestral sense organs. However, non-neural cells comprising the two types of organules are not necessarily homologous. In a recent review of sensillum evolution, Hartenstein (2005) considered that cell recruitment was the original mechanism for

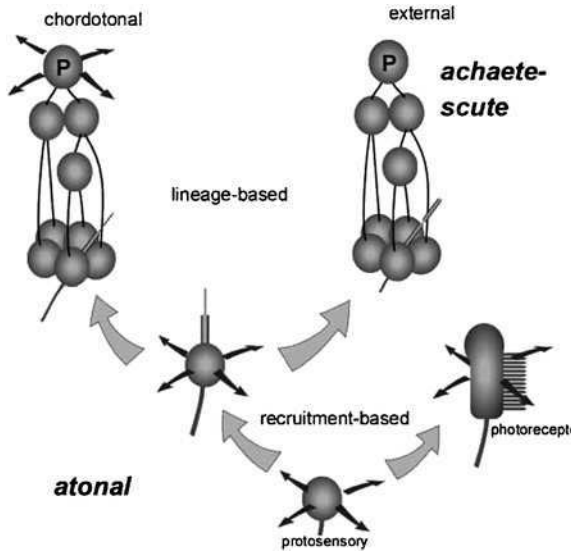



FIG. 4 A possible evolutionary pathway for sensilla and photoreceptors derived from a protosensory organ. Recruitment-based mechanisms (black arrows) are utilized in the *atonal*-expressing sense organs.

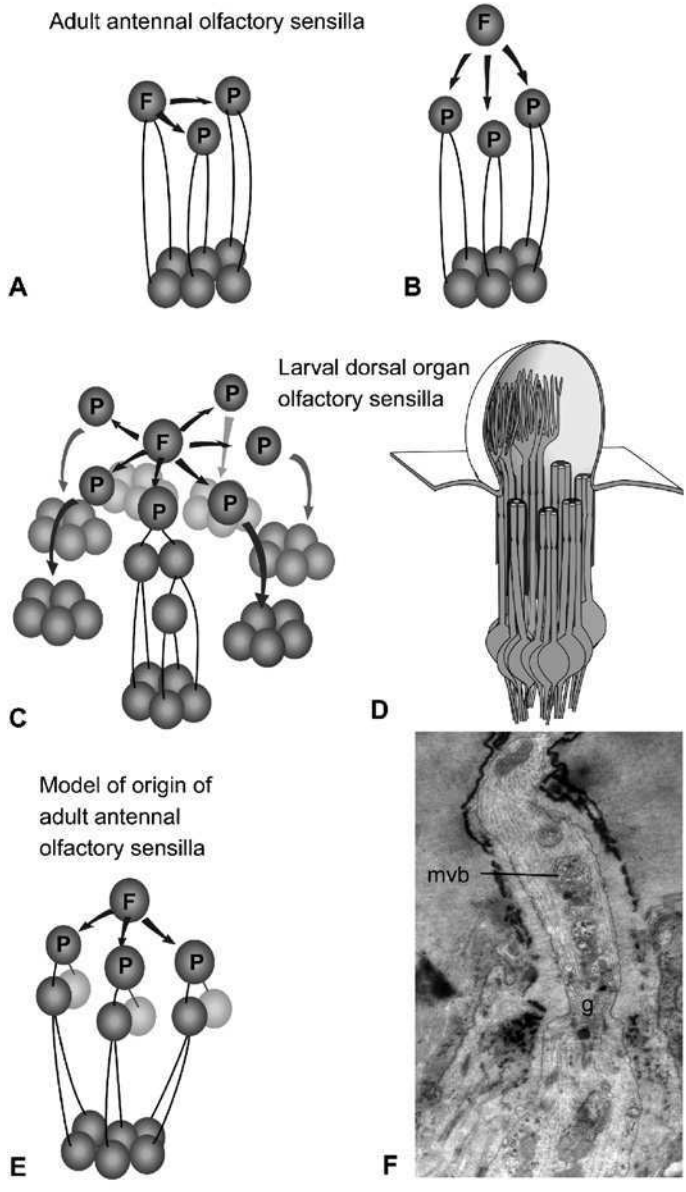
constructing sensilla and that arthropods and some chordates evolved the clonal, lineage-based mechanism. It is likely that such lineage mechanisms either never developed in the precursor to the arthropod eye, or if they did, they regressed and recruitment mechanisms came into play.

### 7.2 LINEAGE OF OLFACTORY SENSILLA

A prominent exception to the lineage-based origin of insect sensilla is the olfactory sensilla on the *Drosophila* antenna that are specified by either *atonal* or *amos* (Ray and Rodrigues, 1995; Reddy *et al*., 1997; Sen *et al*., 2003). After a precursor (founder cell) is singled out, additional cells (pII cells) are recruited, one of which divides to form the trichogen and tormogen cells, another to give additional neurons (Fig. 5). This lineage is atypical and it has been difficult to define (Fig. 5), for example, the origins of the recruited cells are not clear (Sen *et al*., 2003) and the fate of the first-seen founder cell among the three pII cells is not yet clear (Sen *et al*., 2004).

One possibility is that recruitment in the antennal olfactory sensilla is a plesiomorphy, however, the conventional lineage-based formation of the antennal olfactory sensilla of the moth *Antheraea* (Keil and Steiner, 1990; Keil, 1997a) suggests otherwise. Alternatively, a precursor-based lineage could have switched to a recruitment mechanism. This explanation is unlikely because the identities of individual cells in the lineage-based system

rely strongly on the identity of the parent cell and the presence of a sister cell (reviewed by Hartenstein, 2005). An induced cell would possess none of the inherited intrinsic determinants so it is difficult to envisage a process where it could divide and produce daughter cells with morphological characteristics that exactly match conventional, lineage-derived cells.



A second possibility is that the atypical lineage seen in *Drosophila* olfactory sensilla is a case of swapping of competent cells between lineages (Reddy *et al*., 1997; Hartenstein, 2005). During formation of the wing sense organs of *Drosophila*, adjacent organules at the same stage of development can interchange support cells (Hartenstein and Posakony, 1989). The phenomenon relies on two cells of complementary lineage-based identity coming into juxtaposition at just the right time, and is therefore different to induction of a ''naïve'' cell.

A third possibility, incorporating both recruitment and cell-swapping and overcoming the problems associated with either, is suggested from an examination of the larval olfactory organ of Diptera, the dorsal organ. The dorsal organ has an unusual compound morphology, representing an amalgamation of sensilla (Zacharuk and Shields, 1991; Nicastro *et al*., 1998) and is regarded as a reduced and compressed version of the adult antenna (Svacha, 1992; Melzer *et al*., 1999, Merritt, 2005). In the *Drosophila* larva, the somata of the olfactory neurons are in one cluster, however, the dendrites are present in seven clusters, each surrounded by a dendritic sheath and thecogen cell (Fig. 5D). They enter a common sensillum lymph cavity where they branch and come into close contact with the porous cuticle of the dome (Chu-Wang and Axtell, 1971; Singh and Singh, 1984). In mosquito, the seven clusters have been reduced to six, with a corresponding number of tormogen and trichogen cells joining to form the single, common lumen (Zacharuk *et al*., 1971; Nicastro *et al*., 1998), suggesting that the sense organ is a functional and developmental amalgamation of up to seven sensilla. Perhaps the unusual developmental pattern of the adult olfactory sensilla reflects a modified form of the larval compound organs. The component cells could be derived from multiple precursors, each giving rise to a reduced set of cells. For example, the socket and shaft cells are derived from one sense organ precursor, some neurons from another, and thecogen cell plus neuron from another

FIG. 5 Olfactory sensillum lineages. (A) and (B) represent two alternative ways of producing a six-cell olfactory sensillum (based on Ray and Rodrigues, 1995; Reddy *et al*., 1997; Sen *et al*., 2003). In (A), the founder cell recruits ''P'' cells, then acts as a ''P'' cell itself. In (B) the ''F'' cell induces all three ''P'' cells. Its precise fate is not known. In (C), a hypothetical mechanism for formation of the compound dorsal organ sensillum of Diptera is shown. ''P'' cells are recruited by an ''F'' cell and each ''P'' cell gives rise to one of the component sensillum groups that make up the sense organ. (D) is a diagram of the dorsal organ of *Drosophila* showing a cutaway of the seven clusters of neurons and branched dendrites. In (E), the larval dorsal organ mechanism is adapted to show one way that the six-cell adult olfactory sensillum could be produced. F is an electron micrograph of the dendrite of a putative olfactory sensillum located on the ovipositor of the blowfly, *Lucilia cuprina* (Merritt, 1989), showing accumulations of multivesicular bodies (mvb) in the outer dendritic segment.

(Fig. 5E). In evolutionary terms, this type of pattern could have arisen through selective elimination of cells of the conventional larval compound lineage leading to a "mix and match" identity of adult olfactory sensilla. In essence, each adult olfactory sensillum could be a mini-compound sense organ. Such extreme alterations in lineages have been documented; for example, in the embryo of *Drosophila,* the apoptosis of most cells of a "canonical" lineage results in a single multidendritic neuron being all that is left (Orgogozo *et al*., 2002; Lai and Orgogozo, 2004). The model incorporates evidence for recruitment among the "pII" cells of the olfactory sense organs of *Drosophila* (Reddy *et al*., 1997) because the seven ancestral precursors could have been recruited by the same type of mechanism that occurs under control of *ato* in the development of the eye and compound chordotonal organs.

Perhaps the unusual mode of olfactory sensillum development is a plesiomorphy, pointing to a conserved, ancient method of specifying the sensory array of the head that includes olfactory sensilla, ch and photoreceptors, all having in common the use of *atonal* as the proneural gene. The mechanism of lineage-based determination is evolutionarily flexible, especially when combined with recruitment of additional precursors. Further details of the fascinating origin of *Drosophila* antennal olfactory sensilla, and the broad suite of arthropod olfactory sensilla in general, remain to be clarified.

### 7.3 CIRCADIAN RHYTHMICITY IN SENSITIVITY

In *Drosophila* and other insects, clock genes such as *period* (*per*) are expressed in a number of tissues in addition to the LN central pacemaker neurons in the brain (Hall, 1995). Sensory structures of the *Drosophila* eyes, antennae, proboscis, legs and wing show strong, self-sustained diurnal rhythmicity of *per* expression, controlled autonomously and independent of the brain and able to be reset by light (Plautz *et al*., 1997; Cheng and Hardin, 1998; Giebultowicz, 2001). Intriguingly, compound sensory organs such as the eyes and antenna of *Drosophila* show corresponding circadian patterns of peak sensitivity to olfactory stimuli as assessed by electroantennograms (EAGs) and electroretinograms (Chen *et al*., 1992; Krishnan *et al*., 1999). Daily changes in sensitivity of gustatory systems have also been noticed (Blaney *et al*., 1986).

Changed sensitivity in the *Drosophila* olfactory system has also been correlated with altered behavioural responsivity to stimuli (Zhou *et al*., 2005). The cycling of both EAG response and orientation behavioural responses is abolished in mutants for the clock genes *per*, *tim and cry* (Krishnan *et al*., 1999, 2001; Zhou *et al*., 2005). The olfactory neurons themselves were shown to be necessary and sufficient for the olfactory sensitivity rhythms (Tanoue *et al*., 2004). Interestingly, olfactory sensitivity

peaks at around midnight (Krishnan *et al*., 1999; Zhou *et al*., 2005), a time at which locomotory activity is minimal and the flies are asleep (van Swinderen and Andretic, 2003). A number of functional explanations have been put forward to explain this enigmatic rhythmicity. One is that high olfactory sensitivity is important for predator avoidance or opportunistic feeding at a time when the animals are normally asleep (Krishnan *et al*., 1999; Zhou *et al*., 2005). Another is that higher sensory sensitivity at night is more likely to arouse the sleeping brain (van Swinderen and Andretic, 2003). Regarding physiological mechanisms, Tanoue *et al*. (2004) suggested that the sensitivity of the olfactory system could be enhanced or diminished by the regulation of G protein-coupled receptor kinases, arrestins or $IP_3$ proteins, all of which have been associated with desensitization of G proteins after activation by the binding of GPCRs. *Drosophila* possesses several members of the *arrestin* family that could play a role in desensitizing the signalling cascade. Two of them, *arr1* and *arr2,* are expressed in both the eyes and antenna (Merrill *et al*., 2002), while a third, *kurtz*, is expressed in the antenna (Ge *et al*., 2006). Mutations in any of the *arrestin* genes show impaired olfactory orientation behaviour and diminished EAGs (Merrill *et al*., 2005; Ge *et al*., 2006), while *arr1* and *arr2* mutations also cause progressive photoreceptor apoptosis in the visual system as well as altered visual response kinetics (Dolph *et al*., 1993; Alloway and Dolph, 1999).

Any link between *arrestins* and rhythmicity is tentative because synthesis of the visual *arrestin* genes does not cycle in *Drosophila* (Hartman *et al*., 2001), although daily *arrestin* synthesis cycles are present in the honeybee eye (Sasagawa *et al*., 2003), indicating that the process could well be species-specific, possibly related to a species' ecophysiological requirements. However, the demonstration that, in *Drosophila*, *arrestin* regulates light sensitivity by shuttling between the photoreceptor cell body and rhabdomeres (Lee and Montell, 2004) indicates the type of mechanisms that could cyclically affect sensitivity without showing cycling in mRNA synthesis. The non-visual arrestin, *kurtz*, that is expressed in the antenna has not been examined for cyclical expression or intracellular localization. The use in the olfactory and visual systems of common genetic pathways associated with G protein transduction cascades holds promise that the circadian regulation of sensitivity of both systems could utilize similar mechanisms. Given that *per* is expressed in many sensory structures of *Drosophila,* including the gustatory receptors (Plautz *et al*., 1997), they too might show cyclical sensitivity.

A potential link between the visual and olfactory systems is the prevalence of multivesicular bodies (mvbs) in both types of neurons. Ultrastructural examinations have shown insect olfactory sensilla commonly contain high densities of mvbs within the inner or outer dendritic segments (Fig. 5F), in densities not found in other receptor types other than

photoreceptors (Marshall, 1973; Kuhbandner, 1984; Merritt, 1989; Shanbhag *et al*., 2000). Both receptor types have very large membrane surface areas, suggesting that the mvbs are a result of membrane or receptor turnover. In some insects, rhabdomere membrane turnover occurs in daily peaks (Blest, 1988), but not others, including *Drosophila* where turnover is continuous rather than restricted to a particular time of day (Stark *et al*., 1988). Further evidence is required before it is possible to decide whether some aspect of the turnover system is linked to the circadian regulatory pathway shown in these receptor types.

Endogenous, free-running circadian cycling has been seen in other aspects of photoreceptor physiology. Proximal–distal movement of screening pigments occurs within the photoreceptor neurons of the housefly (Pyza and Meinertzhagen, 1997). In addition, the level of visual pigments in *Drosophila* photoreceptors varies on a daily cycle, reaching a maximum before dawn (Stark *et al*., 1988; Chen *et al*., 1992).

It is possible that the peaks of cyclical sensitivity in the sensory systems occur at a time of rest to allow recharging of the sensory systems, for example, membrane or receptor replacement. It will be of great interest to track down the precise targets of the clock genes in insect sensory systems.

## 8 Derivatives of the ciliated organule and the role of the cilium

### 8.1 SCALES AS HOMOLOGS OF SENSILLA

Beside sensilla, Lawrence classified the dermal glands, oenocytes and non-innervated scales as organules (Lawrence, 1966). An examination of scale development in Lepidoptera showed that they are in fact homologous with sensilla in the sense that they show a modified sensillum-like lineage and utilize the same proneural gene as sensilla for the formation of the precursor cell (Fig. 3D). During their development, the scales and innervated sensory structures on the wings and bodies of butterflies express an ortholog of members of the *Drosophila achaete–scute* complex of proneural genes. To make scales, the canonical sensory lineage is modified by apoptosis of some cells of the organule – the potential neuron and sheath cell. The remaining cells differentiate to produce scales and sockets (Galant *et al*., 1998).

### 8.2 DERMAL GLANDS AS HOMOLOGS OF SENSILLA

A subset of insect glands, the group known as class III gland cells according to the classification scheme of Noirot and Quennedey (1974, 1991) show a number of features suggesting that they too might be homologs of

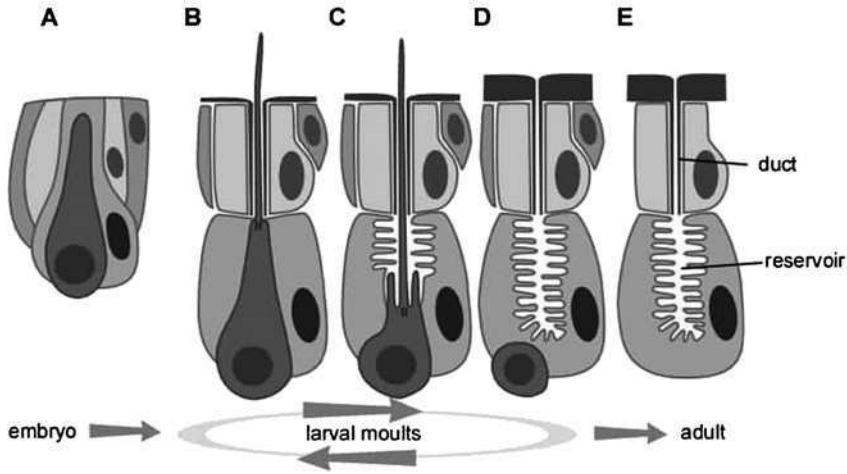



FIG. 6 Diagram of gland development. Four cells make up the gland. The basal cell extends a ciliated process around which the duct is formed. The cell later regresses (D) and degenerates in the adult (E). The secretory cell is the cell immediately wrapping the ciliated cell (modified from Quennedey, 1998).

sensilla. Type III gland cells are the basal cells of multicellular units associated with the hypodermis, thus the units can also be called dermal glands, distinguishing them from internal, tubular glands such as salivary glands. The dermal glands are composed of one to many cells forming an extracellular reservoir and a duct that opens to the exterior (Fig. 6). They are always associated with the hypodermis. Some internal glands are, in fact, dermal glands because much of the insect internal reproductive system and gut is ectodermal in origin and secretes modified cuticle. For example, the venom glands of bees and wasps are dermal glands that secrete into specialized cuticle-lined pouches or sacs (Billen, 1990).

While extensive literature is available on the ultrastructure of dermal glands, developmental studies are relatively few (reviewed by Quennedey, 1998). A survey of the developmental and structural features of insect dermal glands reveals a number of convincing parallels with sense organs: (1) both are derived from a single precursor cell that delaminates from the epidermis and divides several times (Selman and Kafatos, 1975; Sreng and Quennedey, 1976; Sreng, 1985, 1998; Quennedey, 1991), (2) the basal cell of the gland lineage gives rise to a ciliated dendrite-like structure that forms the mould for the ductule and later regresses (reviewed in Sreng and Quennedey, 1976; Quennedey, 1998), (3) in both, one or more cells release secretion into a microvillate extracellular lumen (Noirot and Quennedey, 1974), (4) endoreplication of component cells is seen in both organule types, (5) apoptosis of a subset of cells can occur in both organules after

their developmental role is complete in the adult stage (e.g. Keil, 1978; Sreng, 1998), (6) dermal glands of the bug *Rhodnius* use the same ''lateral inhibition'' spacing mechanisms as sense organs (Wigglesworth, 1953), and (7) the induction of an ectopic adult moult through hormonal manipulation of *Rhodnius* causes the dermal glands to produce ill-formed cuticular hairs resembling sense organs (Wigglesworth, 1953).

One of the most compelling pieces of evidence for dermal gland/sense organ homology is the presence in both of a ciliated process of the basalmost cell. In glands, the cilium is ephemeral and appears to be required as a cellular template for formation of the ductule (Barbier, 1975; Selman and Kafatos, 1975; Happ and Happ, 1977; Bitsch, 1981) (Fig. 6). However, in one contradictory instance, the ductules of the female accessory glands of *Rhodnius* form around non-ciliated extensions of the basal cell (Lococo and Huebner, 1980). The cilia of sensory organules have a similar role, forming the template for secretion of the dendritic sheath, in addition to their role in sensory transduction.

A clear intermediate between sensillum and gland is seen in the dipluran apterygote, *Campodea chardardi* (Jacquemin and Bareth, 1981) (Fig. 7). Organules on the sternites of the male possess a hollow cuticular bristle with a pore at the tip. Before and immediately after each moult, the dendrites of three neurons are present, two extending to the tip and one terminating at its base in a tubular body, so the organules conform to the morphology of a classic gustatory/mechanosensory hair (Zacharuk, 1980). However, in the intermoult period the dendrites degenerate and one of the support cells produces a voluminous secretion that is released from the tip of the bristle. The growth/degeneration cycle is repeated at each moult, indicating that the dendrites are required for regeneration of the hair, probably as a template for the channel within the hair shaft, but otherwise the organule appears to be purely secretory. The phallic glands of the silverfish show a number of similar characteristics (Bitsch, 1990). Additional examples include the silk-secreting spinnerets of spiders (Bond, 1994; Craig, 1997), the silk-producing bristles of empidid flies (Young and Merritt, 2003), and the silk glands of webspinners (Nagashima *et al*., 1991), the latter two appearing to be derived from gustatory sensilla (Young and Merritt, 2003). Based on the broad variety of putative functions of the gland secretions and different locations of the glands, it appears that glands have repeatedly and independently evolved from sense organs in different lineages of insects.

Sreng and Quennedey (1976) and Quennedey (1998) pointed out a number of similarities between sense organs and glands but the relationship has never been fully explored. From the available evidence, it is tempting to speculate that dermal glandular units are homologs of sensilla, however, lineage-tracing and gene expression studies are needed to test this hypothesis.

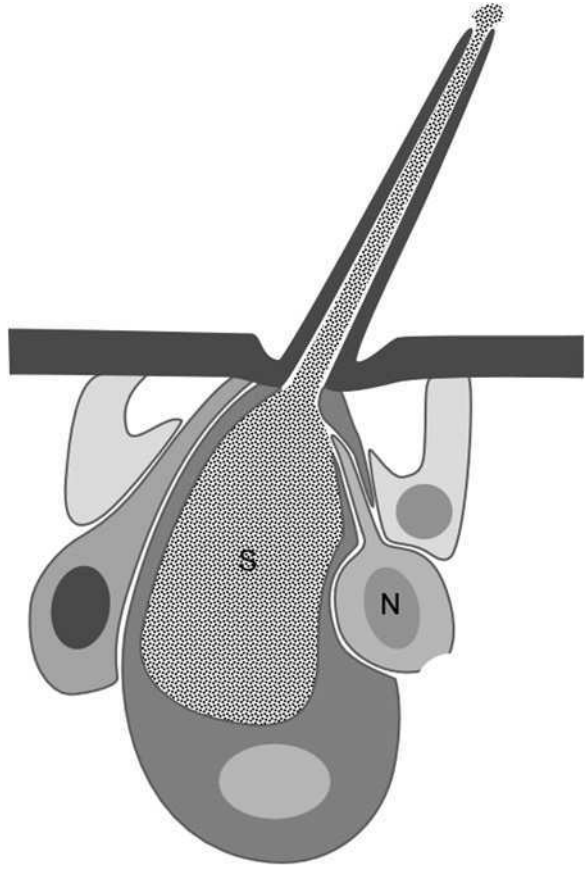



FIG. 7 Organule intermediate between gland and sensillum found in the dipluran apterygote, *Campodea chardardi* (adapted from Jacquemin and Bareth, 1981). N: neuron-like cell. S: secretion.

## 8.3 MOULTING AND CILIATED CELLS

Periodic moulting means a new, immature exoskeleton has to be ready beneath the old one at each moult. In sense organs, the cilium plays a role in cyclical morphogenesis occurring at moulting, in addition to its role in sensory transduction. During the period between apolysis (separation of old cuticle from new) and ecdysis (the moult itself), when two cuticular layers are present, sensory function is maintained by dendritic contact with the outer cuticle until just before ecdysis when the dendrite breaks away and reforms its terminus under the newly secreted hair (Gnatzy and Tautz, 1977; Gnatzy, 1978) (Fig. 8). The need for this cyclical recapitulation of development also applies to dermal glands because at each moult the

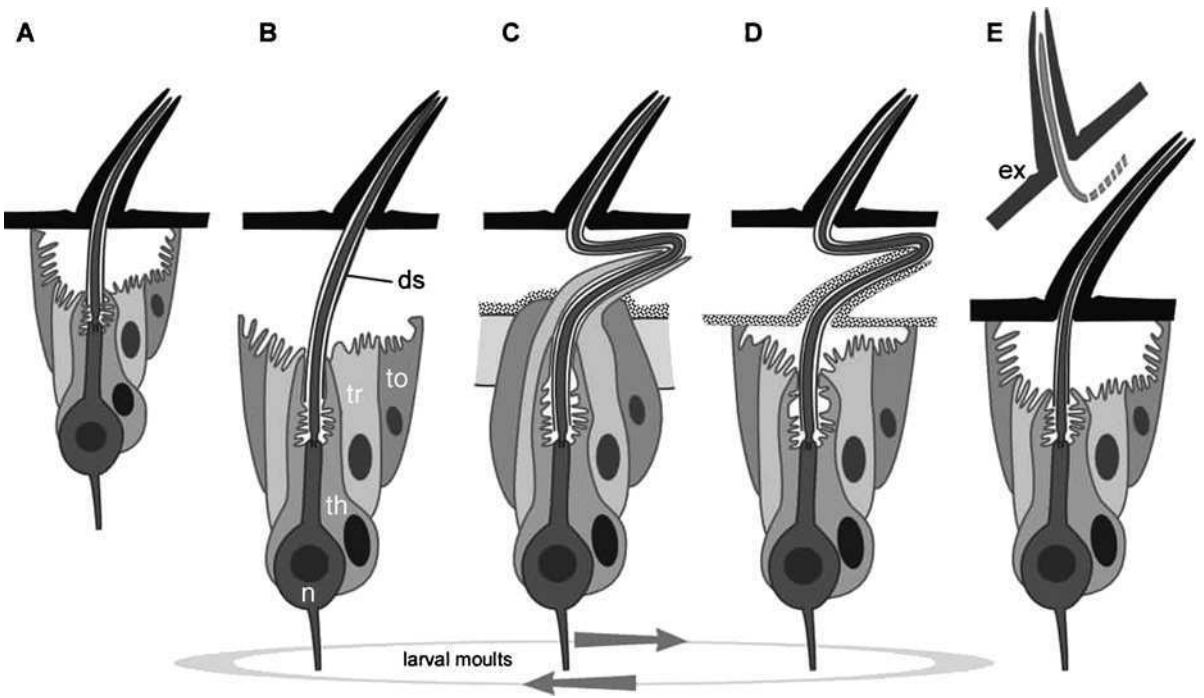



FIG. 8 The moulting cycle of a contact chemoreceptor sensillum. Apolysis (B) is followed by outgrowth of the trichogen cell (tr) around the dendrite and dendritic sheath (ds) (C). After the new hair is formed around the trichogen sprout (D), the dendrite breaks and the old exoskeleton (ex) is cast off (derived from Hansen and Hansen-Delkeskamp, 1983, and Seidl, 1991). For clarity, only one sensory neuron is represented. To, tormogen cell; th, thecogen cell; n, neuron.

conducting canal and end apparatus are shed with the old cuticle, meaning a new canal must be secreted around the ciliary extension of the basal cell.

In the sensory nervous system, the cilium has two functions, sensory transduction and regeneration at the moult. In glands, it appears to have only one function, regeneration. So, if we think of organules as hypodermal organs that must re-establish their cuticular contacts or components at each moult, then the cilium could be viewed as a structure whose fundamental role is in recapitulation rather than being an absolute requirement for sensory transduction.

The two distinct roles of the sensory cilium might shed light on the evolution of the multidendritic neurons. The fact that two types of sensory neurons can be derived from a single lineage is puzzling. Why are some neurons ciliated and others unciliated? The two types are genetically closely related. As mentioned above, a single lineage can give rise to both ciliated (type I) and unciliated (type II) neurons. The *Drosophila hamlet* gene modulates this aspect of neuronal identity: within a single lineage, loss of *hamlet* function leads to the es neuron taking on the md neuron fate and, *vice versa*, gain of function transforms the md neuron into an es neuron (Moore *et al.*, 2002). In the moulting context, a fundamental difference between them is that the type II neuron has lost its association with the cuticle because the soma drifts away from the parent organule and the dendrites ramify beneath the hypodermis. Perhaps, these neurons have then lost the constraint of regrowth at a moult, becoming free to modify the dendritic segment by losing the cilium and dramatically expanding the dendritic branches.

The argument appears to fall down when applied to ch organs because they too are internalized, having lost a direct ciliary connection to the cuticle in amphinematic ch. I suggest that the cilium is retained in this particular modality of sensillum because it is an essential component of the transduction apparatus and, despite the cilium no longer being required for moulting, it is required for transduction (see Section 3.2.4). It follows that md neurons should be derived from sensory neurons whose transduction mechanisms were not reliant on the cilium *per se*, such as gustatory or olfactory sense organs. Interestingly, one thing that gustatory and md neurons have in common is that they are the only types of neurons that utilize the Deg/ENaC *ppk* gene family of ion channels (Adams *et al.*, 1998; Liu *et al.*, 2003a), and the TRPA1 family member, *painless*, is expressed in both md neurons and gustatory neurons where it mediates the response to noxious heat and isothiocyanate, respectively (Tracey *et al.*, 2003; Al-Anzi *et al.*, 2006). Perhaps md neurons and gustatory neurons have a common ancestor.

## 9 Conclusions

In this review, I have examined the diversity of sensillum types and the diversity of transduction mechanisms used by them. Our knowledge of the

insect sensory system has benefited extensively from the genomics approach when combined with classical forward genetics in *Drosophila*, and one of my aims has been to place this information in the context of the evolution of insect organules. Rather than a comprehensive coverage, I have tended to use information gained from *Drosophila* developmental genetics to discuss a few pertinent issues, including the ancient homology between photoreceptors and sensilla, a more recent derivation of glands from sensilla, and a re-evaluation of the role of the cilium that places an emphasis on its role in moulting rather than sensory transduction. An emerging story that is not yet complete is the common developmental mechanisms of the sense organs that are clustered in large groups on the head: the photoreceptors, olfactory sensilla and ch. They all show attributes making them different to the organules encountered on other parts of the body, suggesting that the evolution of the head sensory apparatus is somehow unique in insects, perhaps because it follows an ancient developmental pattern that is resistant to change. One of the benefits of the comparative approach is that it places the insights gained from *Drosophila* into the context of other, less derived insects and future genomics initiatives may help reveal the details of evolutionary patterns among insect organules.

## Acknowledgements

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## References

Adams, C. M., Anderson, M. G., Motto, D. G., Price, M. P., Johnson, W. A. and Welsh, M. J. (1998). *Ripped pocket* and *pickpocket*, novel *Drosophila* DEG/ENaC subunits expressed in early development and in mechanosensory neurons. *J. Cell Biol.* **140**, 143–152.

Ainsley, J. A., Pettus, J. M., Bosenko, D., Gerstein, C. E., Zinkevich, N., Anderson, M. G., Adams, C. M., Welsh, M. J. and Johnson, W. A. (2003). Enhanced locomotion caused by loss of the *Drosophila* DEG/ENaC protein *Pickpocket1*. *Curr. Biol.* **13**, 1557–1563.

Al-Anzi, B., Tracey, W. D. and Benzer, S. (2006). Response of *Drosophila* to wasabi is mediated by *painless*, the fly homolog of mammalian TRPA1/ANKTM1. *Curr. Biol.* **16**, 1034–1040.

Alloway, P. G. and Dolph, P. J. (1999). A role for the light-dependent phosphorylation of visual arrestin. *Proc. Natl. Acad. Sci. USA* **96**, 6072–6077.

Altner, H. and Loftus (1985). Ultrastructure and function of insect thermo- and hygroreceptors. *Annu. Rev. Entomol.* **30**, 273–295.

Altner, H. and Prillinger, L. (1980). Ultrastructure of invertebrate chemo-, thermo-, and hygroreceptors and its functional significance. *Int. Rev. Cytol.* **67**, 69–139.

Amrein, H. and Thorne, N. (2005). Gustatory perception and behavior in *Drosophila melanogaster*. *Curr. Biol.* **15**, R673–R684.

Anderson, M. and Finlayson, L. H. (1978). Topography and electrical activity of peripheral neurons in the abdomen of the tsetse fly (*Glossina*) in relation to abdominal distension. *Physiol. Entomol.* **3**, 157–167.

Arikawa, K., Eguchi, E., Yoshida, A. and Aoki, K. (1980). Multiple extra-ocular photoreceptive areas on genitalia of butterfly *Papilio xuthus*. *Nature* **288**, 700–702.

Avidor-Reiss, T., Maer, A. M., Koundakjian, E., Polyanovsky, A., Keil, T., Subramaniam, S. and Zuker, C. S. (2004). Decoding cilia function: defining specialized genes required for compartmentalized cilia biogenesis. *Cell* **117**, 527–539.

Baker, J. D., Adhikarakunnathu, S. and Kernan, M. J. (2004). Mechanosensory-defective, male-sterile *unc* mutants identify a novel basal body protein required for ciliogenesis in *Drosophila*. *Development* **131**, 3411–3422.

Barbier, R. (1975). Differentiation of ciliary structures and organization of canal during organogenesis of the colleterial glands of *Galleria mellonella* Lepidoptera Pyralidae. *J. Microsc. Biol. Cell* **24**, 315–326.

Baumgartner, S., Littleton, J. T., Broadie, K., Bhat, M. A., Harbecke, R., Lengyel, J. A., Chiquet-Ehrismann, R., Prokop, A. and Bellen, H. J. (1996). A *Drosophila* neurexin is required for septate junction and blood-nerve barrier formation and function. *Cell* **87**, 1059–1068.

Benton, R., Sachse, S., Michnick, S. W. and Vosshall, L. B. (2006). Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors *in vivo*. *PLOS Biol.* **4**, 240–257.

Bertrand, N., Castro, D. S. and Guillemot, F. (2002). Proneural genes and the specification of neural cell types. *Nat. Rev. Neurosci.* **3**, 517–530.

Billen, J. (1990). The sting bulb gland in *Myrmecia* and *Nothomyrmecia* (Hymenoptera: Formicidae): A new exocrine gland in ants. *Int. J. Insect Morphol. Embryol.* **19**, 133–139.

Bitsch, J. (1981). Ultrastructural modifications of the glandular epithelium of the receptaculum seminis in *Thermobia domestica* (Insecta: Thysanura) during the moulting period. *Cell Tissue Res.* **220**, 99–114.

Bitsch, J. (1990). Ultrastructure of the phallic glands of the firebrat *Thermobia domestica* Packard Thysanura Lepismatidae. *Int. J. Insect Morphol. Embryol.* **19**, 65–78.

Blaney, W. M., Schoonhoven, L. M. and Simmonds, M. S. J. (1986). Sensitivity variations in insect chemoreceptors: a review. *Experientia* **42**, 13–19.

Blest, A. D. (1988). The turnover of phototransductive membrane in compound eyes and ocelli. *Adv. Insect Physiol.* **20**, 1–53.

Blomquist, G. and Vogt, R. (2003). *Insect Pheromone Biochemistry and Molecular Biology: The Biosynthesis and Detection of Pheromones and Plant Volatiles*. Amsterdam, Netherlands: Elsevier Academic Press.

Boekhoff-Falk, G. (2005). Hearing in *Drosophila*: Development of Johnston's organ and emerging parallels to vertebrate ear development. *Dev. Dyn.* **232**, 550–558.

Bond, J. E. (1994). Seta-spigot homology and silk production in first instar *Antrodiaetus unicolor* spiderlings (Araneae: Antrodiaetidae). *J. Arachnol.* **22**, 19–22.

Bray, S. and Amrein, H. (2003). A putative *Drosophila* pheromone receptor expressed in male-specific taste neurons is required for efficient courtship. *Neuron* **39**, 1019–1029.

Brewster, R. and Bodmer, R. (1995). Origin and specification of type II sensory neurons in *Drosophila*. *Development* **121**, 2923–2936.

Caldwell, J. C. and Eberl, D. F. (2002). Towards a molecular understanding of *Drosophila* hearing. *J. Neurobiol.* **53**, 172–189.

Caldwell, J. C., Miller, M. M., Wing, S., Soll, D. R. and Eberl, D. F. (2003). Dynamic analysis of larval locomotion in *Drosophila* chordotonal organ mutants. *Proc. Natl. Acad. Sci. USA* **100**, 16053–16058.

Chen, D. M., Christianson, J. S., Sapp, R. J. and Stark, W. S. (1992). Visual receptor cycle in normal and *period* mutant *Drosophila*: Microspectrophotometry, electrophysiology, and ultrastructural morphometry. *Vis. Neurosci.* **9**, 125–135.

Cheng, Y. Z. and Hardin, P. E. (1998). *Drosophila* photoreceptors contain an autonomous circadian oscillator that can function without period mRNA cycling. *J. Neurosci.* **18**, 741–750.

Chown, S. L. and Terblanche, J. S. (2007). Physiological diversity in insects: ecological and evolutionary contexts. *Adv. Insect Physiol.* **33**, 51–155.

Chung, Y. D., Zhu, J., Han, Y. and Kernan, M. J. (2001). nompA encodes a PNS-specific, ZP domain protein required to connect mechanosensory dendrites to sensory structures. *Neuron* **29**, 415–428.

Chu-Wang, I. and Axtell, R. (1971). Fine structure of the dorsal organ of the house fly larva, *Musca domestica* L. *Z. Zellforsch.* **117**, 17–34.

Clyne, P. J., Warr, C. G., Freeman, M. R., Lessing, D., Kim, J. H. and Carlson, J. R. (1999). A novel family of divergent seven-transmembrane proteins: Candidate odorant receptors in *Drosophila*. *Neuron* **22**, 327–338.

Clyne, P. J., Warr, C. G. and Carlson, J. R. (2000). Candidate taste receptors in *Drosophila*. *Science* **287**, 1830–1834.

Craig, C. L. (1997). Evolution of arthropod silks. *Annu. Rev. Entomol.* **42**, 231–267.

Crouau, Y. (1983). Ultrastructure study of the 9 + 0 type cilia of insect and crustacean chordotonal sensilla. Generalisation of the hypothesis on the motility of 9 + 0 type cilia. *J. Submicrosc. Cytol.* **15**, 295–299.

de Bruyne, M. and Warr, C. G. (2006). Molecular and cellular organization of insect chemosensory neurons. *Bioessays* **28**, 23–34.

del Alamo, D., Terriente, J. and Diaz-Benjumea, F. J. (2002). Spitz/EGFr signalling via the Ras/MAPK pathway mediates the induction of bract cells in *Drosophila* legs. *Development* **129**, 1975–1982.

Dethier, V. G. (1963). *The Physiology of Insect Senses*. London, UK: Methuen and Co. Ltd.

Dethier, V. G. (1976). *The Hungry Fly: A Physiological Study of the Behavior Associated with Feeding*. Cambridge, MA: Harvard University Press.

Dobritsa, A. A., van der Goes van Naters, W., Warr, C. G., Steinbrecht, R. A. and Carlson, J. R. (2003). Integrating the molecular and cellular basis of odor coding in the *Drosophila* antenna. *Neuron* **37**, 827–841.

Dolph, P. J., Ranganathan, R., Colley, N. J., Hardy, R. W., Socolich, M. and Zuker, C. S. (1993). Arrestin function in inactivation of G-protein coupled receptor rhodopsin *in vivo*. *Science* **260**, 1910–1916.

Dubruille, R., Laurencon, A., Vandaele, C., Shishido, E., Coulon_Bublex, M., Swoboda, P., Couble, P., Kernan, M. and Durand, B. (2002). *Drosophila* regulatory factor X is necessary for ciliated sensory neuron differentiation. *Development* **129**, 5487–5498.

Dunipace, L., Meister, S., McNealy, C. and Amrein, H. (2001). Spatially restricted expression of candidate taste receptors in the *Drosophila* gustatory system. *Curr. Biol.* **11**, 822–835.

Eberl, D. F., Duyk, G. M. and Perrimon, N. (1997). A genetic screen for mutations that disrupt an auditory response in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **94**, 14837–14842.

Eberl, D. F., Hardy, R. W. and Kernan, M. J. (2000). Genetically similar transduction mechanisms for touch and hearing in *Drosophila*. *J. Neurosci.* **20**, 5981–5988.
Edwards, J. (1998). Sir Vincent Wigglesworth and the coming of age of insect development. *Int. J. Dev. Biol.* **42**, 471–473.
Elliott, S. L., Cullen, C. F., Wrobel, N., Kernan, M. J. and Ohkura, H. (2005). EB1 is essential during *Drosophila* development and plays a crucial role in the integrity of chordotonal mechanosensory organs. *Mol. Biol. Cell* **16**, 891–901.
Elstob, P., Brodu, V. and Gould, A. (2001). Spalt-dependent switching between two cell fates that are induced by the *Drosophila* EGF receptor. *Development* **128**, 723–732.
Field, L. H. and Matheson, T. (1998). Chordotonal organs of insects. *Adv. Insect Physiol.* **27**, 1–228.
Finlayson, L. H. (1968). Proprioceptors in the invertebrates. *Symp. Zool. Soc. Lond.* **23**, 217–249.
Fishilevich, E. and Vosshall, L. B. (2005). Genetic and functional subdivision of the *Drosophila* antennal lobe. *Curr. Biol.* **15**, 1548–1553.
Foelix, R. F., Stocker, R. F. and Steinbrecht, R. A. (1989). Fine structure of a sensory organ in the arista of *Drosophila melanogaster* and some other dipterans. *Cell Tissue Res.* **258**, 277–287.
Freeman, M. (1997). Cell determination strategies in the *Drosophila* eye. *Development* **124**, 261–270.
French, A. (1988). Transduction mechanisms of mechanosensilla. *Annu. Rev. Entomol.* **33**, 39–58.
Galant, R., Skeath, J. B., Paddock, S., Lewis, D. L. and Carroll, S. B. (1998). Expression pattern of a butterfly *Achaete–Scute* homolog reveals the homology of butterfly wing scales and insect sensory bristles. *Curr. Biol.* **8**, 807–813.
Galindo, K. and Smith, D. P. (2001). A large family of divergent *Drosophila* odorant-binding proteins expressed in gustatory and olfactory sensilla. *Genetics* **159**, 1059–1072.
Gao, Q. and Chess, A. (1999). Identification of candidate *Drosophila* olfactory receptors from genomic DNA sequence. *Genomics* **60**, 31–39.
Ge, H., Krishnan, P., Liu, L. Z., Krishnan, B., Davis, R. L., Hardin, P. E. and Roman, G. (2006). A *Drosophila* nonvisual *arrestin* is required for the maintenance of olfactory sensitivity. *Chem. Senses* **31**, 49–62.
Giebultowicz, J. M. (2001). Peripheral clocks and their role in circadian timing: insights from insects. *Phil. Trans. R. Soc. Lond. B.* **356**, 1791–1799.
Gnatzy, W. (1978). Development of the filiform hairs on the cerci of *Gryllus bimaculatus* Deg. (Saltatoria, Gryllidae). *Cell Tissue Res.* **187**, 1–24.
Gnatzy, W. and Tautz, J. (1977). Sensitivity of an insect mechanoreceptor during molting. *Physiol. Entomol.* **2**, 279–288.
Goldman, A. L., van Naters, W. v. d. G., Lessing, D., Warr, C. G. and Carlson, J. R. (2005). Coexpression of two functional odor receptors in one neuron. *Neuron* **45**, 661–666.
Gong, Z., Son, W., Chung, Y. D., Kim, J., Shin, D. W., McClung, C. A., Lee, Y., Lee, H. W., Chang, D. J., Kaang, B. K., Cho, H., Oh, U., Hirsh, J., Kernan, M. J. and Kim, C. (2004). Two interdependent TRPV channel subunits, *inactive* and *Nanchung*, mediate hearing in *Drosophila*. *J. Neurosci.* **24**, 9059–9066.
Goodman, M. B. and Schwarz, E. M. (2003). Transducing touch in *Caenorhabditis elegans*. *Annu. Rev. Physiol.* **65**, 429–452.
Gopfert, M. C. and Robert, D. (2000). Nanometre-range acoustic sensitivity in male and female mosquitoes. *Proc. R. Soc. Lond. B. Biol. Sci.* **267**, 453–457.

Gopfert, M. C. and Robert, D. (2001). Active auditory mechanics in mosquitoes. *Proc. R. Soc. Lond. B. Biol. Sci.* **268**, 333–339.

Gopfert, M. C. and Robert, D. (2003). Motion generation by *Drosophila* mechanosensory neurons. *Proc. Natl. Acad. Sci. USA* **100**, 5514–5519.

Gopfert, M. C., Humphris, A. D., Albert, J. T., Robert, D. and Hendrich, O. (2005). Power gain exhibited by motile mechanosensory neurons in *Drosophila* ears. *Proc. Natl. Acad. Sci. USA* **102**, 325–330.

Goulding, S. E., zur Lage, P. and Jarman, A. P. (2000). *Amos*, a proneural gene for *Drosophila* olfactory sense organs that is regulated by *lozenge*. *Neuron* **25**, 69–78.

Gregory, S. L. and Brown, N. H. (1998). *kakapo*, a gene required for adhesion between and within cell layers in *Drosophila*, encodes a large cytoskeletal linker protein related to plectin and dystrophin. *J. Cell Biol.* **143**, 1271–1282.

Grueber, W. B. and Truman, J. W. (1999). Development and organization of a nitric-oxide-sensitive peripheral neural plexus in larvae of the moth, *Manduca sexta*. *J. Comp. Neurol.* **404**, 127–141.

Grueber, W. B., Graubard, K. and Truman, J. W. (2001). Tiling of the body wall by multidendritic sensory neurons in *Manduca sexta*. *J. Comp. Neurol.* **440**, 271–283.

Hall, J. (1995). Tripping along the trail to the molecular mechanisms of biological clocks. *Trends Neurosci.* **18**, 230–240.

Hallem, E. A. and Carlson, J. R. (2004). The odor coding system of *Drosophila*. *Trends Genet.* **20**, 453–459.

Hallem, E. A., Ho, M. G. and Carlson, J. R. (2004). The molecular basis of odor coding in the *Drosophila* antenna. *Cell* **117**, 965–979.

Hallem, E. A., Dahanukar, A. and Carlson, J. R. (2006). Insect odor and taste receptors. *Annu. Rev. Entomol.* **51**, 113–135.

Halter, D. A., Urban, J., Rickert, C., Ner, S. S., Ito, K., Travers, A. A. and Technau, G. M. (1995). The homeobox gene *repo* is required for the differentiation and maintenance of glia function in the embryonic nervous system of *Drosophila melanogaster*. *Development* **121**, 317–332.

Han, Y. G., Kwok, B. H. and Kernan, M. J. (2003). Intraflagellar transport is required in *Drosophila* to differentiate sensory cilia but not sperm. *Curr. Biol.* **13**, 1679–1686.

Hansen, K. and Hansen-Delkeskamp, E. (1983). The development of taste and tactile hairs in the pharate fly *Protophormia terraenovae* (Diptera, Calliphoridae) and in the embryonal cricket *Acheta domestica* (Orthopteroidea, Ensifera). *Zoomorphology* **102**, 241–259.

Happ, G. M. and Happ, C. M. (1977). Cytodifferentiation in accessory-glands of *Tenebrio molitor* 3. Fine-structure of spermathecal accessory-gland in the pupa. *Tissue & Cell* **9**, 711–732.

Hartenstein, V. (2005). Development of insect sensilla. In: *Comprehensive Molecular Insect Science*, Vol. 1 (eds Lawrence, I. G., Kostas, I. and Sarjeet, S. G.), pp. 379–419. Elsevier, Amsterdam, Netherlands.

Hartenstein, V. and Posakony, J. W. (1989). Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. *Development* **107**, 389–405.

Hartman, S. J., Menon, I., Haug-Collet, K. and Colley, N. J. (2001). Expression of rhodopsin and arrestin during the light-dark cycle in *Drosophila*. *Mol. Vis.* **7**, 95–100.

Hekmat-Scafe, D. S., Scafe, C. R., McKinney, A. J. and Tanouye, M. A. (2002). Genome-wide analysis of the odorant-binding protein gene family in *Drosophila melanogaster*. *Genome Res.* **12**, 1357–1369.

Henke, K. (1953). Uber Zelldifferenzierung im Integument der Insekten und ihre Bedingungen. *J. Embryol. Exp. Morphol.* **1**, 217–226.

Hill, C. A., Fox, A. N., Pitts, R. J., Kent, L. B., Tan, P. L., Chrystal, M. A., Cravchik, A., Collins, F. H., Robertson, H. M. and Zwiebel, L. J. (2002). G protein-coupled receptors in *Anopheles gambiae*. *Science* **298**, 176–178.

Howard, J. and Bechstedt, S. (2004). Hypothesis: A helix of ankyrin repeats of the NOMPC-TRP ion channel is the gating spring of mechanoreceptors. *Curr. Biol.* **14**, R224–R226.

Huang, M. L., Hsu, C. H. and Chien, C. T. (2000). The proneural gene *amos* promotes multiple dendritic neuron formation in the *Drosophila* peripheral nervous system. *Neuron* **25**, 57–67.

Inbal, A., Volk, T. and Salzberg, A. (2004). Recruitment of ectodermal attachment cells via an EGFR-dependent mechanism during the organogenesis of *Drosophila* proprioceptors. *Dev. Cell* **7**, 241–250.

Jacquemin, G. and Bareth, C. (1981). Ultrastructure of glandular and sensory bristles of the 1st abdominal sternite of *Campodea chardardi* (Insecta: Diplura) males: Modifications connected with molting. *Int. J. Insect Morphol. Embryol.* **10**, 463–482.

Jacquin-Joly, E. and Merlin, C. (2004). Insect olfactory receptors: contributions of molecular biology to chemical ecology. *J. Chem. Ecol.* **30**, 2359–2397.

Jarman, A. P. (2002). Studies of mechanosensation using the fly. *Hum. Mol. Genet.* **11**, 1215–1218.

Jarman, A. P., Grell, E. H., Ackerman, L., Jan, L. Y. and Jan, Y. N. (1994). *atonal* is the proneural gene for *Drosophila* photoreceptors. *Nature* **369**, 398–400.

Jefferis, G. S. X. E. and Hummel, T. (2006). Wiring specificity in the olfactory system. *Sem. Cell Dev. Biol.* **17**, 50–65.

Jones, B. W., Fetter, R. D., Tear, G. and Goodman, C. S. (1995). *glial cells missing*: a genetic switch that controls glial versus neuronal fate. *Cell* **82**, 1013–1023.

Jones, W. D., Nguyen, T. A. T., Kloss, B., Lee, K. J. and Vosshall, L. B. (2005). Functional conservation of an insect odorant receptor gene across 250 million years of evolution. *Curr. Biol.* **15**, R119–R121.

Kaissling, K. E. (1996). Peripheral mechanisms of pheromone reception in moths. *Chem. Senses* **21**, 257–268.

Keil, T. A. (1978). Die makrochaeten auf dem thorax von *Calliphora vicina* Robineau–Desvoidy (Calliphoridae, Diptera). *Zoomorphol.* **90**, 151–180.

Keil, T. A. (1997a). Functional morphology of insect mechanoreceptors. *Microsc. Res. Tech.* **39**, 506–531.

Keil, T. A. (1997b). Comparative morphogenesis of sensilla: a review. *Int. J. Insect Morphol. Embryol.* **26**, 151–160.

Keil, T. and Steinbrecht, R. (1984). Mechanosensitive and olfactory sensilla of insects. In: *Insect Ultrastructure*, Vol. 2 (eds King, R. and Akai, H.), pp. 477–516. New York, USA: Plenum Press.

Keil, T. and Steiner, C. (1990). Morphogenesis of the antenna of the male silkmoth, *Antheraea polyphemus*. II. Differential mitoses of "dark" precursor cells create the anlagen of sensilla. *Tissue & Cell* **22**, 705–720.

Kernan, M., Cowan, D. and Zuker, C. (1994). Genetic dissection of mechanosensory transduction: mechanoreception-defective mutations of *Drosophila*. *Neuron* **12**, 1195–1206.

Kim, M. S., Repp, A. and Smith, D. P. (1998). LUSH odorant-binding protein mediates chemosensory responses to alcohols in *Drosophila melanogaster*. *Genetics* **150**, 711–721.

Kim, J., Chung, Y. D., Park, D. Y., Choi, S., Shin, D. W., Soh, H., Lee, H. W., Son, W., Yim, J., Park, C. S. *et al.* (2003). A TRPV family ion channel required for hearing in *Drosophila*. *Nature* **424**, 81–84.

Krieger, J., Vonnickischrosenegk, E., Mameli, M., Pelosi, P. and Breer, H. (1996). Binding proteins from the antennae of *Bombyx mori*. *Insect Biochem. Molec. Biol.* **26**, 297–307.

Krishnan, B., Dryer, S. E. and Hardin, P. E. (1999). Circadian rhythms in olfactory responses of *Drosophila melanogaster*. *Nature* **400**, 375–378.

Krishnan, B., Levine, J. D., Lynch, M. K., Dowse, H. B., Funes, P., Hall, J. C., Hardin, P. E. and Dryer, S. E. (2001). A new role for cryptochrome in a *Drosophila* circadian oscillator. *Nature* **411**, 313–317.

Kuhbandner, B. (1984). Ultrastructure and ontogeny of the hair sensilla on the funicle of *Calliphora erythrocephala* (Insecta, Diptera). I. The basiconical sensilla. *Zoomorphol.* **104**, 373–385.

Kuhbandner, B. (1985). Ultrastructure and ontogeny of the double-walled sensilla on the funicle of *Calliphora erythrocephala* Meigen (Diptera: Calliphoridae). *Int. J. Insect Morphol. Embryol.* **14**, 227–242.

Lai, E. C. and Orgogozo, V. (2004). A hidden program in *Drosophila* peripheral neurogenesis revealed: fundamental principles underlying sensory organ diversity. *Dev. Biol.* **269**, 1–17.

Laissue, P. P., Reiter, C., Hiesinger, P. R., Halter, S., Fischbach, K. F. and Stocker, R. F. (1999). Three-dimensional reconstruction of the antennal lobe in *Drosophila melanogaster*. *J. Comp. Neurol.* **405**, 543–552.

Larsson, M. C., Domingos, A. I., Jones, W. D., Chiappe, M. E., Amrein, H. and Vosshall, L. B. (2004). *Or83b* encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. *Neuron* **43**, 703–714.

Lawrence, P. A. (1966). Development and determination of hairs and bristles in the milkweed bug, *Oncopeltus fasciatus* (Lygaeidae, Hemiptera). *J. Cell Sci.* **1**, 475–498.

Lee, S. J. and Montell, C. (2004). Light-dependent translocation of visual arrestin regulated by the NINAC myosin III. *Neuron* **43**, 95–103.

Lee, Y., Lee, Y., Lee, J., Bang, S., Hyun, S., Kang, J., Hong, S. T., Bae, E., Kaang, B. K. and Kim, J. (2005). *Pyrexia* is a new thermal transient receptor potential channel endowing tolerance to high temperatures in *Drosophila melanogaster*. *Nat. Genet.* **37**, 305–310.

Liedtke, W. and Kim, C. (2005). Functionality of the TRPV subfamily of TRP ion channels: add mechano-TRP and osmo-TRP to the lexicon!. *Cell. Mol. Life Sci.* **62**, 2985–3001.

Lin, H. P., Mann, K. J., Starostina, E., Kinser, R. D. and Pikielny, C. W. (2005). A *Drosophila* DEG/ENaC channel subunit is required for male response to female pheromones. *Proc. Natl. Acad. Sci. USA* **102**, 12831–12836.

Liu, L., Leonard, A. S., Motto, D. G., Feller, M. A., Price, M. P., Johnson, W. A. and Welsh, M. J. (2003a). Contribution of *Drosophila* DEG/ENaC genes to salt taste. *Neuron* **39**, 133–146.

Liu, L., Yermolaieva, O., Johnson, W. A., Abboud, F. M. and Welsh, M. J. (2003b). Identification and function of thermosensory neurons in *Drosophila* larvae. *Nat. Neurosci.* **6**, 267–273.

Lococo, D. and Huebner, E. (1980). The development of the female accessory gland in the insect *Rhodnius prolixus*. *Tissue & Cell* **12**, 795–813.

McKenna, M., Monte, P., Helfand, S. L., Woodard, C. and Carlson, J. (1989). A simple chemosensory response in *Drosophila* and the isolation of *acj* mutants in which it is affected. *Proc. Natl. Acad. Sci. USA* **86**, 8118–8122.

Marshall, A. T. (1973). Vesicular structures in the dendrites of an insect olfactory receptor. *Tissue & Cell* **5**, 233–241.

Martinez-Campos, M., Basto, R., Baker, J., Kernan, M. and Raff, J. W. (2004). The *Drosophila* pericentrin-like protein is essential for cilia/flagella function, but appears to be dispensable for mitosis. *J. Cell Biol.* **165**, 673–683.

Melo, A. C. A., Rutzler, M., Pitts, R. J. and Zwiebel, L. J. (2004). Identification of a chemosensory receptor from the yellow fever mosquito, *Aedes aegypti*, that is highly conserved and expressed in olfactory and gustatory organs. *Chem. Senses* **29**, 403–410.

Melzer, R. R., Sprenger, J., Nicastro, D. and Smola, U. (1999). Larva-adult relationships in an ancestral dipteran: A re-examination of sensillar pathways across the antenna and leg anlagen of *Chaoborus crystallinus* (DeGeer, 1776; Chaoboridae). *Dev. Genes Evol.* **209**, 103–112.

Melzer, R. R., Michalke, C. and Smola, U. (2000). Walking on insect paths? Early ommatidial development in the compound eye of the ancestral crustacean, *Triops cancriformis*. *Naturwissenschaften* **87**, 308–311.

Merrill, C. E., Riesgo-Escovar, J., Pitts, R. J., Kafatos, F. C., Carlson, J. R. and Zwiebel, L. J. (2002). Visual arrestins in olfactory pathways of *Drosophila* and the malaria vector mosquito *Anopheles gambiae*. *Proc. Natl. Acad. Sci. USA* **99**, 1633–1638.

Merrill, C. E., Sherertz, T. M., Walker, W. B. and Zwiebel, L. J. (2005). Odorant-specific requirements for arrestin function in *Drosophila* olfaction. *J. Neurobiol.* **63**, 15–28.

Merritt, D. J. (1989). The cercal sensilla of the blowfly, *Lucilia cuprina*. II. Structure of the enveloping cells and basal regions of the sensory dendrites. *Tissue & Cell* **21**, 281–298.

Merritt, D. J. (1997). Transformation of external sensilla to chordotonal sensilla in the *cut* mutant of *Drosophila* assessed by single cell marking in the embryo and larva. *Microsc. Res. Tech.* **39**, 492–505.

Merritt, D. J. (2005). The evolution and development of the dipteran nervous system. In: *The Evolutionary Biology of Flies* (eds Yeates, D. K. and Wiegmann, B. M.). New York, USA: Columbia University Press.

Merritt, D. J. and Whitington, P. M. (1995). Central projections of sensory neurons in the *Drosophila* embryo correlate with sensory modality, soma position and proneural gene function. *J. Neurosci.* **15**, 1755–1767.

Miyako, Y., Arikawa, K. and Eguchi, E. (1993). Ultrastructure of the extraocular photoreceptor in the genitalia of a butterfly, *Papilio xuthus*. *J. Comp. Neurol.* **327**, 458–468.

Montell, C. (2005). *Drosophila* TRP channels. *Pflugers Arch.* **451**, 19–28.

Moore, A. W., Jan, L. Y. and Jan, Y. N. (2002). *hamlet*, a binary genetic switch between single- and multiple-dendrite neuron morphology. *Science* **297**, 1355–1358.

Moulins, M. (1976). Ultrastructure of chordotonal organs. In: *Structure and Function of Proprioceptors in the Invertebrates* (ed. Mill, P.), pp. 387–426. London, UK: Chapman and Hall.

Nagashima, T., Niwa, N., Okajima, S. and Nonaka, T. (1991). Ultrastructure of silk gland of webspinners *Oligotoma japonica* (Insecta: Embioptera). *Cytologia* **56**, 679–685.

Nakagawa, T., Sakurai, T., Nishioka, T. and Touhara, K. (2005). Insect sex-pheromone signals mediated by specific combinations of olfactory receptors. *Science* **307**, 1638–1642.

Nicastro, D., Melzer, R. R., Hruschka, H. and Smola, U. (1998). Evolution of small sense organs: Sensilla on the larval antennae traced back to the origin of the Diptera. *Naturwissenschaften* **85**, 501–505.

Nighorn, A., Simpson, P. and Morton, D. (2001). The novel guanylyl cyclase MsGC-I is strongly expressed in higher-order neuropils in the brain of *Manduca sexta*. *J. Exp. Biol.* **204**, 305–314.

Nishikawa, M., Yokohari, F. and Ishibashi, T. (1995). Central projections of the antennal cold receptor neurons and hygroreceptor neurons of the cockroach *Periplaneta americana*. *J. Comp. Neurol.* **361**, 165–176.

Niwa, N., Hiromi, Y. and Okabe, M. (2004). A conserved developmental program for sensory organ formation in *Drosophila melanogaster*. *Nat. Genet.* **36**, 293–297.

Noirot, C. and Quennedey, A. (1974). Fine-structure of insect epidermal glands. *Annu. Rev. Entomol.* **19**, 61–80.

Noirot, C. and Quennedey, A. (1991). Glands, gland cells, glandular units: some comments on terminology and classification. *Ann. Soc. Entomol. Fr.* **27**, 123–128.

Orgogozo, V., Schweisguth, F. and Bellaiche, Y. (2002). Binary cell death decision regulated by unequal partitioning of *Numb* at mitosis. *Development* **129**, 4677–4684.

Pesavento, P. A., Stewart, R. J. and Goldstein, L. S. B. (1994). Characterization of the Klp68d kinesin-like protein in *Drosophila*: possible roles in axonal transport. *J. Cell Biol.* **127**, 1041–1048.

Pitts, R. J., Fox, A. N. and Zwiebel, L. J. (2004). A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector *Anopheles gambiae*. *Proc. Natl. Acad. Sci. USA* **101**, 5058–5063.

Plautz, J. D., Kaneko, M., Hall, J. C. and Kay, S. A. (1997). Independent photoreceptive circadian clocks throughout *Drosophila*. *Science* **278**, 1632–1635.

Prokop, A., Uhler, J., Roote, J. and Bate, M. (1998). The *kakapo* mutation affects terminal arborization and central dendritic sprouting of *Drosophila* motorneurons. *J. Cell Biol.* **143**, 1283–1294.

Pyza, E. and Meinertzhagen, I. A. (1997). Circadian rhythms in screening pigment and invaginating organelles in photoreceptor terminals of the housefly's first optic neuropile. *J. Neurobiol.* **32**, 517–529.

Quennedey, A. (1991). The moulting process of perennial class 3 gland cells during the postembryonic development of two heterometabolous insects: Blaberus (Dictyoptera) and Dysdercus (Heteroptera). *Annales de la Societe Entomologique de France* **27**, 143–161.

Quennedey, A. (1998). Insect epidermal gland cells: ultrastructure and morphogenesis. In: *Microscopic Anatomy of Invertebrates*, Vol. 11. Insecta (eds Locke, M. and Harrison, F. W.), pp. 177–207. Wiley-Liss, Inc.

Ray, K. and Rodrigues, V. (1995). Cellular events during development of the olfactory sense organs in *Drosophila melanogaster*. *Dev. Biol.* **167**, 426–438.

Ray, K., Perez, S. E., Yang, Z., Xu, J., Ritchings, B. W., Steller, H. and Goldstein, L. S. (1999). Kinesin-II is required for axonal transport of choline acetyltransferase in *Drosophila*. *J. Cell Biol.* **147**, 507–518.

Reddy, G., Gupta, B., Ray, K. and Rodrigues, V. (1997). Development of the *Drosophila* olfactory sense organs utilizes cell–cell interactions as well as lineage. *Development* **124**, 703–712.

Rice, M. J., Galun, R. and Finlayson, L. H. (1973). Mechanotransduction in insect neurones. *Nature* **241**, 286–288.

Robert, D. and Gopfert, M. C. (2002). Acoustic sensitivity of fly antennae. *J. Insect Physiol.* **48**, 189–196.
Robertson, H. M., Warr, C. G. and Carlson, J. R. (2003). Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **100**, 14537–14542.
Rogers, S. M. and Newland, P. L. (2003). The neurobiology of taste in insects. *Adv. Insect Physiol.* **31**, 141–204.
Rogers, M. E., Krieger, J. and Vogt, R. G. (2001). Antennal SNMPs (sensory neuron membrane proteins) of Lepidoptera define a unique family of invertebrate CD36-like proteins. *J. Neurobiol.* **49**, 47–61.
Roper, K., Gregory, S. L. and Brown, N. H. (2002). The 'Spectraplakins': cytoskeletal giants with characteristics of both spectrin and plakin families. *J. Cell Sci.* **115**, 4215–4225.
Rosenzweig, M., Brennan, K. M., Tayler, T. D., Phelps, P. O., Patapoutian, A. and Garrity, P. A. (2005). The *Drosophila* ortholog of vertebrate TRPA1 regulates thermotaxis. *Genes Dev.* **19**, 419–424.
Rusten, T. E., Cantera, R., Urban, J., Technau, G., Kafatos, F. C. and Barrio, R. (2001). Spalt modifies EGFR-mediated induction of chordotonal precursors in the embryonic PNS of *Drosophila* promoting the development of oenocytes. *Development* **128**, 711–722.
Sarpal, R. and Ray, K. (2002). Dynamic expression pattern of kinesin accessory protein in *Drosophila*. *J. Biosci.* **27**, 479–487.
Sarpal, R., Todi, S. V., Sivan-Loukianova, E., Shirolikar, S., Subramanian, N., Raff, E. C., Erickson, J. W., Ray, K. and Eberl, D. F. (2003). *Drosophila* KAP interacts with the kinesin II motor subunit KLP64D to assemble chordotonal sensory cilia, but not sperm tails. *Curr. Biol.* **13**, 1687–1696.
Sasagawa, H., Narita, R., Kitagawa, Y. and Kadowaki, T. (2003). The expression of genes encoding visual components is regulated by a circadian clock, light environment and age in the honeybee (*Apis mellifera*). *Eur. J. Neurosci.* **17**, 963–970.
Schmitz, H. and Wasserthal, L. T. (1993). Antennal thermoreceptors and wing-thermosensitivity of heliotherm butterflies: their possible role in thermoregulatory behavior. *J. Insect Physiol.* **39**, 1007–1019.
Schrader, S. and Merritt, D. J. (2000). Central projections of *Drosophila* sensory neurons in the transition from embryo to larva. *J. Comp. Neurol.* **425**, 34–44.
Scott, K., Brady, R., Cravchik, A., Morozov, P., Rzhetsky, A., Zuker, C. and Axel, R. (2001). A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell* **104**, 661–673.
Seidl, S. (1991). Structure and differentiation of the sensilla of the ventral sensory field on the maxillary palps of *Periplaneta americana* (Insecta, Blattodea), paying special attention to the ciliogenesis of the sensory cells. *Zoomorphology* **111**, 35–47.
Selman, K. and Kafatos, F. C. (1975). Differentiation in cocoonase-producing silkmoth galea: Ultrastructural studies. *Dev. Biol.* **46**, 132–150.
Sen, A., Reddy, G. V. and Rodrigues, V. (2003). Combinatorial expression of Prospero, Seven-up, and Elav identifies progenitor cell types during sense-organ differentiation in the *Drosophila* antenna. *Dev. Biol.* **254**, 79–92.
Sen, A., Kuruvilla, D., Pinto, L., Sarin, A. and Rodrigues, V. (2004). Programmed cell death and context dependent activation of the EGF pathway regulate gliogenesis in the *Drosophila* olfactory system. *Mech. Dev.* **121**, 65–78.
Shanbhag, S. R., Muller, B. and Steinbrecht, R. A. (1999). Atlas of olfactory organs of *Drosophila melanogaster* 1. Types, external organization, innervation and distribution of olfactory sensilla. *Int. J. Insect Morphol. Embryol.* **28**, 377–397.

Shanbhag, S. R., Muller, B. and Steinbrecht, R. A. (2000). Atlas of olfactory organs of *Drosophila melanogaster* 2. Internal organization and cellular architecture of olfactory sensilla. *Arthropod Str. Dev.* **29**, 211–229.

Shanbhag, S. R., Smith, D. P. and Steinbrecht, R. A. (2005). Three odorant-binding proteins are co-expressed in sensilla trichodea of *Drosophila melanogaster*. *Arthropod Struct. Dev.* **34**, 153–165.

Singh, R. N. and Singh, K. (1984). Fine structure of the sensory organs of *Drosophila melanogaster* Meigen larva (Diptera: Drosophilidae). *Int. J. Insect. Morphol. Embryol.* **13**, 255–273.

Slifer, E. H. (1970). The structure of arthropod chemoreceptors. *Annu. Rev. Entomol.* **15**, 121–142.

Sreng, L. (1985). Ultrastructure of the glands producing sex pheromones of the male *Nauphoeta cinerea* (Insecta, Dictyoptera). *Zoomorphology* **105**, 133–142.

Sreng, L. (1998). Apoptosis-inducing brain factors in maturation of an insect sex pheromone gland during differentiation. *Differentiation* **63**, 53–58.

Sreng, L. and Quennedey, A. (1976). Role of a temporary ciliary structure in the morphogenesis of insect glands. An electron microscope study of the tergal glands of male *Blattella germanica* L. (Dictyoptera, Blattellidae). *J. Ultrastruct. Res.* **56**, 78–95.

Steinbrecht, R. A., Ozaki, M. and Ziegelberger, G. (1992). Immunocytochemical localization of pheromone-binding protein in moth antennae. *Cell Tissue Res.* **270**, 287–302.

Stark, W. S., Sapp, R. and Schilly, D. (1988). Rhabdomere turnover and rhodopsin cycle: Maintenance of retinula cells in *Drosophila melanogaster*. *J. Neurocytol.* **17**, 499–509.

Stocker, R. F. (1994). The organization of the chemosensory system in *Drosophila melanogaster*: a review. *Cell Tissue Res.* **275**, 3–26.

Svacha, P. (1992). What are and what are not imaginal discs: reevaluation of some basic concepts (Insecta, Holometabola). *Dev. Biol.* **154**, 101–117.

Tanoue, S., Krishnan, P., Krishnan, B., Dryer, S. E. and Hardin, P. E. (2004). Circadian clocks in antennal neurons are necessary and sufficient for olfaction rhythms in *Drosophila*. *Curr. Biol.* **14**, 638–649.

Tepass, U., Theres, C. and Knust, E. (1990). *crumbs* encodes an EGF-like protein expressed on apical membranes of *Drosophila* epithelial cells and required for organization of epithelia. *Cell* **61**, 787–799.

Thorne, N., Chromey, C., Bray, S. and Amrein, H. (2004). Taste perception and coding in *Drosophila*. *Curr. Biol.* **14**, 1065–1079.

Thurm, U. (1964). Mechanoreceptors in the cuticle of the honey bee: fine structure and stimulus mechanism. *Science* **145**, 1063–1065.

Thurm, U., Erler, G., Godde, J., Kastrup, H., Keil, T. h., Volker, W. and Vohwinkel, B. (1983). Cilia specialized for mechanoreception. *J. Submicrosc. Cytol.* **15**, 151–155.

Todi, S. V., Sharma, Y. and Eberl, D. F. (2004). Anatomical and molecular design of the *Drosophila* antenna as a flagellar auditory organ. *Microsc. Res. Tech.* **63**, 388–399.

Todi, S. V., Franke, J. D., Kiehart, D. P. and Eberl, D. F. (2005). Myosin VIIA defects, which underlie the Usher 1B syndrome in humans, lead to deafness in *Drosophila*. *Curr. Biol.* **15**, 862–868.

Toh, Y. (1985). Structure of campaniform sensilla on the haltere of *Drosophila* prepared by cryofixation. *J. Ultrastruct. Res.* **93**, 92–100.

Tracey, W. D., Wilson, R. I., Laurent, G. and Benzer, S. (2003). *Painless*, a *Drosophila* gene essential for nociception. *Cell* **113**, 261–273.

van Swinderen, B. and Andretic, R. (2003). Arousal in *Drosophila*. *Behav. Processes* **64**, 133–144.
Vervoort, M., Merritt, D., Ghysen, A. and Dambly-Chaudiere, C. (1997). Genetic basis of the formation and identity of type I and type II neurons in *Drosophila* embryos. *Development* **124**, 2819–2828.
Vogt, R. G. and Riddiford, L. M. (1981). Pheromone-binding and inactivation by moth antennae. *Nature* **293**, 161–163.
Vosshall, L. (2003). Diversity and expression of odorant receptors in *Drosophila* (eds Blomquist, G. and Vogt, R.)Insect Pheromone Biochemistry and Molecular Biology: The Biosyntehsis and Detection of Pheromones and Plant Volatiles, pp. 567–591. Amsterdam, Netherlands: Elsevier Academic Press.
Vosshall, L. B., Amrein, H., Morozov, P. S., Rzhetsky, A. and Axel, R. (1999). A spatial map of olfactory receptor expression in the *Drosophila* antenna. *Cell* **96**, 725–736.
Walker, R. G., Willingham, A. T. and Zuker, C. S. (2000). A *Drosophila* mechanosensory transduction channel. *Science* **287**, 2229–2234.
Wang, Z., Singhvi, A., Kong, P. and Scott, K. (2004). Taste representations in the *Drosophila* brain. *Cell* **117**, 981–991.
Weber, T., Gopfert, M. C., Winter, H., Zimmermann, U., Kohler, H., Meier, A., Hendrich, O., Rohbock, K., Robert, D. and Knipper, M. (2003). Expression of prestin-homologous solute carrier (SLC26) in auditory organs of nonmammalian vertebrates and insects. *Proc. Natl. Acad. Sci. USA* **100**, 7690–7695.
Wigglesworth, V. (1953). The origin of sensory neurones in an insect, *Rhodnius prolixus* (Hemiptera). *Q. J. Microsc. Sci.* **94**, 93–112.
Williams, D. W. and Truman, J. W. (2004). Mechanisms of dendritic elaboration of sensory neurons in *Drosophila*: Insights from *in vivo* time lapse. *J. Neurosci.* **24**, 1541–1550.
Wolfrum, U. (1990). Actin filaments: the main components of the scolopale in insect sensilla. *Cell & Tissue Research* **261**, 85–96.
Wolfrum, U. (1991a). Tropomyosin is co-localized with the actin filaments of the scolopale in insect sensilla. *Cell Tissue Res.* **265**, 11–17.
Wolfrum, U. (1991b). Centrin-like and a-actinin-like immunoreactivity in the ciliary rootlets of insect sensilla. *Cell Tissue Res.* **266**, 231–238.
Wolfrum, U. (1992). Cytoskeletal elements in arthropod sensilla and mammalian photoreceptors. *Biol. Cell* **76**, 373–381.
Wolfrum, U. (1997). Cytoskeletal elements in insect sensilla. *Int. J. Insect Morphol. Embryol.* **26**, 191–203.
Wright, B. R. (1976). Limb and wing receptors in insects, chelicerates and myriapods. In: *Structure and Function of Proprioceptors in the Invertebrates* (ed. Mill, P.), pp. 323–386. London, UK: Chapman and Hall.
Xu, A., Park, S. K., D'Mello, S., Kim, E., Wang, Q. and Pikielny, C. W. (2002). Novel genes expressed in subsets of chemosensory sensilla on the front legs of male *Drosophila melanogaster*. *Cell Tissue Res.* **307**, 381–392.
Xu, P. X. (2005). A *Drosophila* OBP required for pheromone signaling. *Science* **310**, 798–799.
Xu, P. X., Atkinson, R., Jones, D. N. M. and Smith, D. P. (2005). *Drosophila* OBP *Lush* is required for activity of pheromone-sensitive neurons. *Neuron* **45**, 193–200.
Yack, J. E. (2004). The structure and function of auditory chordotonal organs in insects. *Microsc. Res. Tech.* **63**, 315–337.
Yack, J. E. and Roots, B. I. (1992). The metathoracic wing-hinge chordotonal organ of an atympanate moth, *Actias luna* (Lepidoptera, Saturniidae): A light-microscopic and electron-microscopic study. *Cell Tissue Res.* **267**, 455–471.

Young, J. and Merritt, D. (2003). The ultrastructure and function of the silk-producing basitarsus in the Hilarini (Diptera: Empididae). *Arthropod Struct. Dev.* **32**, 157–165.

Zacharuk, R. Y. (1980). Ultrastructure and function of insect chemosensilla. *Annu. Rev. Entomol.* **25**, 27–47.

Zacharuk, R. Y., Yin, L. R. S. and Blue, S. G. (1971). Fine structure of the antenna and its sensory cone in larvae of *Aedes aegypti* (L). *J. Morphol.* **135**, 273–297.

Zacharuk, R. Y. and Shields, V. D. (1991). Sensilla of immature insects. *Annu. Rev. Entomol.* **36**, 331–354.

Zhou, X. J., Yuan, C. Y. and Guo, A. K. (2005). *Drosophila* olfactory response rhythms require clock genes but not pigment dispersing factor or lateral neurons. *J. Biol. Rhythms* **20**, 237–244.

zur Lage, P. and Jarman, A. P. (1999). Antagonism of EGFR and Notch signalling in the reiterative recruitment of *Drosophila* adult chordotonal sense organ precursors. *Development* **126**, 3149–3157.

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