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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 °C), especially by inoculation if they become wet (freezing at c. −3.7–−4.5 °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-1α

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

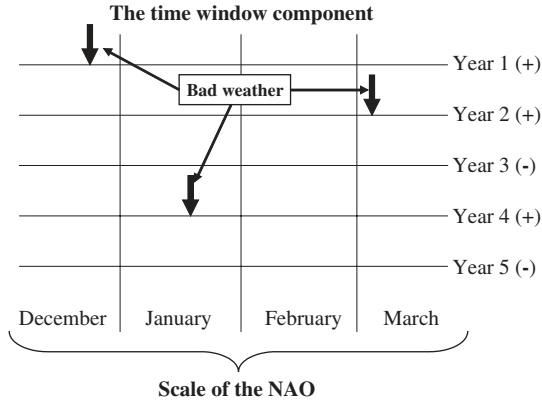


FIG. 1 Climate indices such as the North Atlantic Oscillation (NAO) integrate a variety of weather variables across spatial and temporal scales. Here, poor weather in years two, three, and four takes place in different months. However, the sign of the climate index (in this case NAO) indicates that these 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).

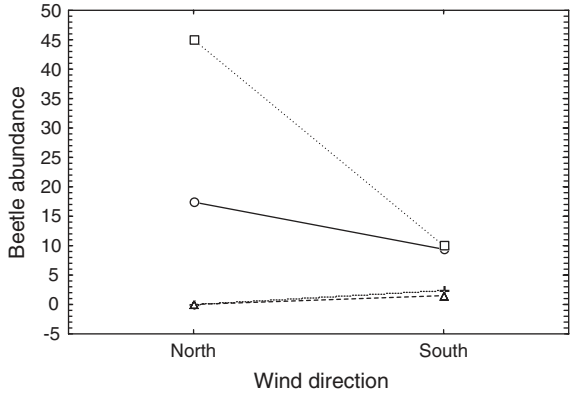


FIG. 2 Interaction plot of mean numbers of adult *Bothrometopus brevis* weevils active at a site on sub-Antarctic Heard Island for each combination of weather conditions prevailing at the site over the course of a summer, including the two major wind directions (north or south) and either no precipitation (○), 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).

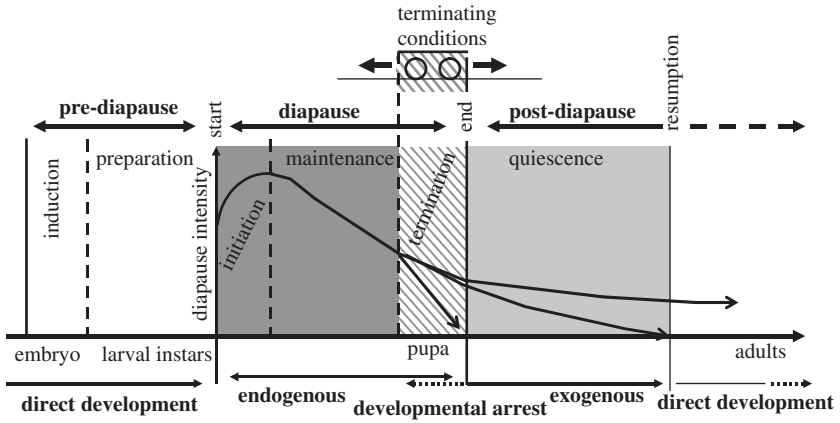


FIG. 3 Schematic depiction of the major phases of diapause, *viz*. pre-diapause, diapause, and post-diapause, as defined by Koštál (2006). Further division into subphases, *viz*. induction, preparation, initiation, maintenance, termination, and quiescence is indicated by vertical lines. Redrawn with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

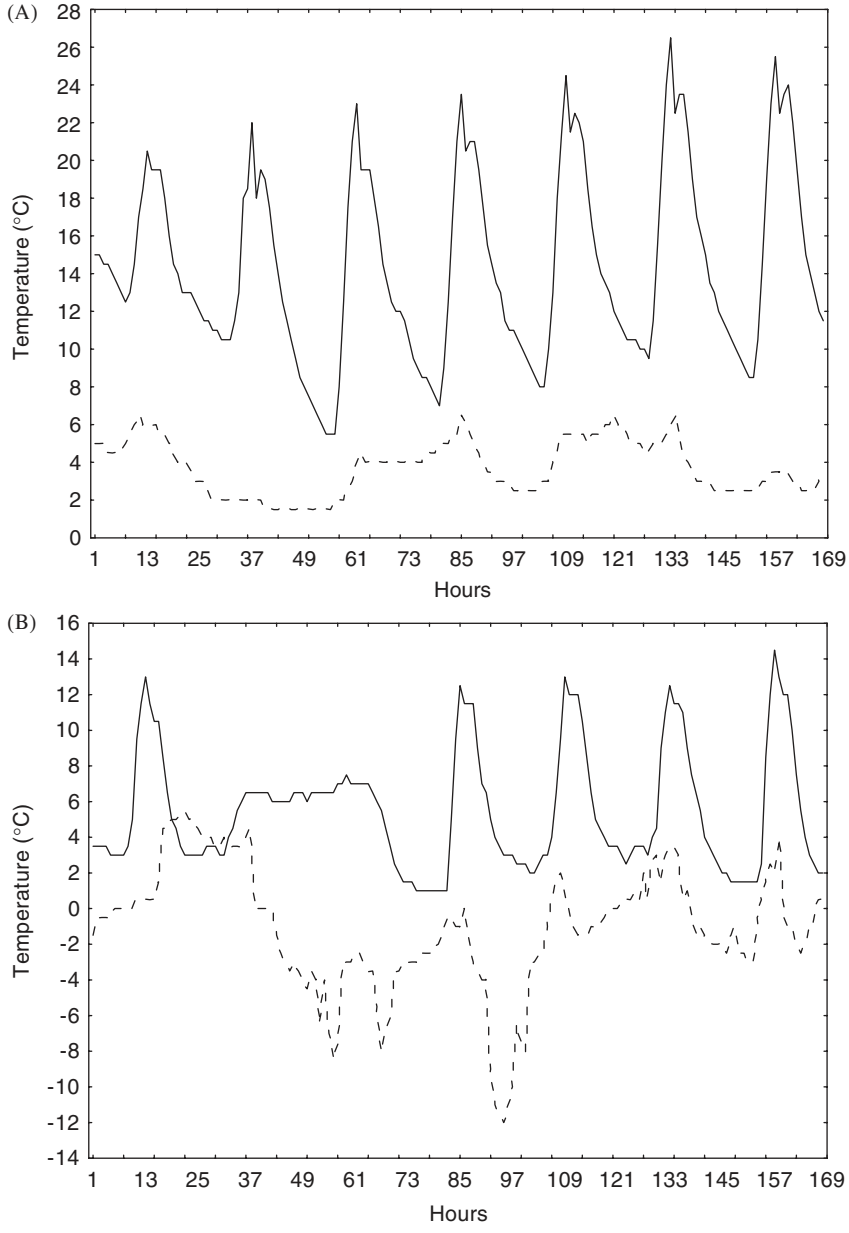


FIG. 4 Hourly temperatures at the soil surface over a week long period in August 2002 for (A) a sea-level site at Lambert's Bay on the west coast of South Africa (solid line) and a sea-level site at sub-Antarctic Marion Island (dashed line), and (B) a site (Sneeukop) at 1960 m above sea level 50 km distant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

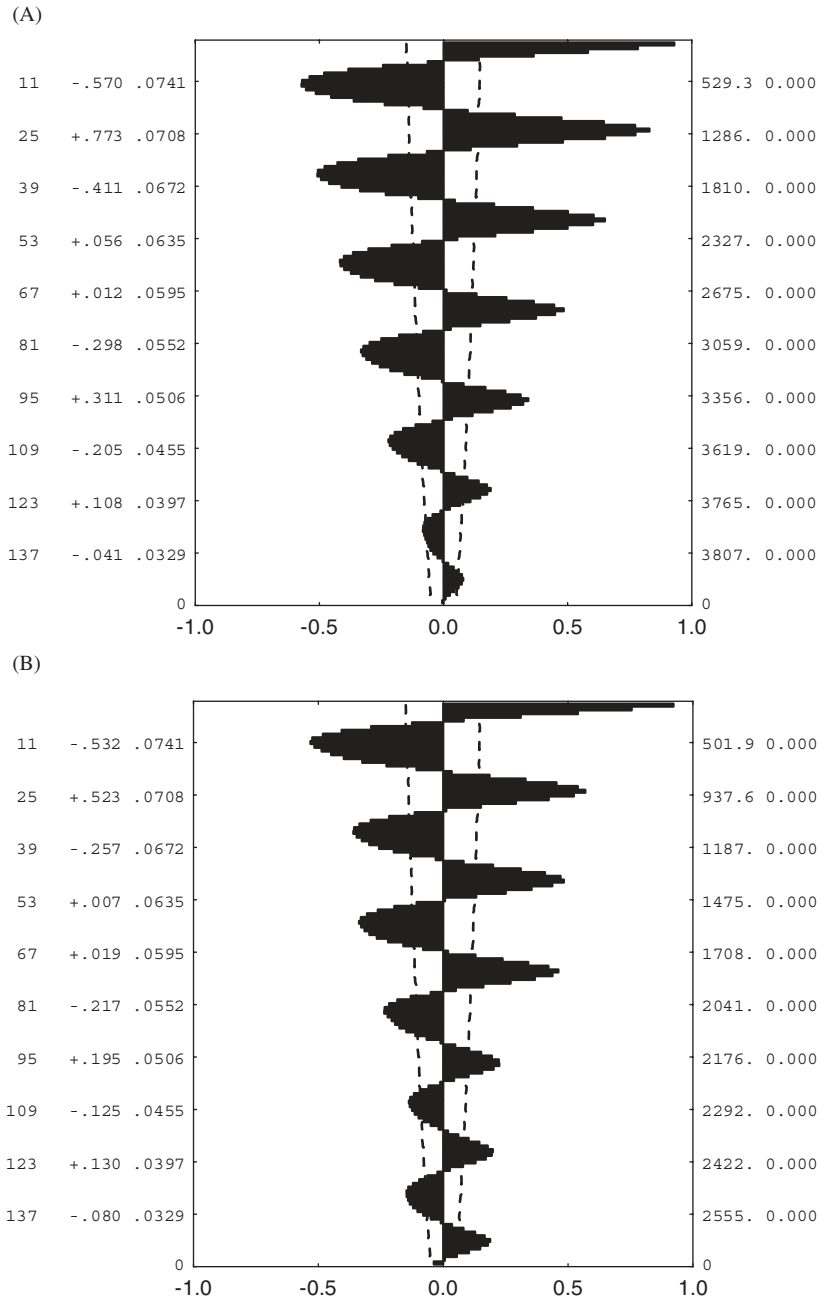
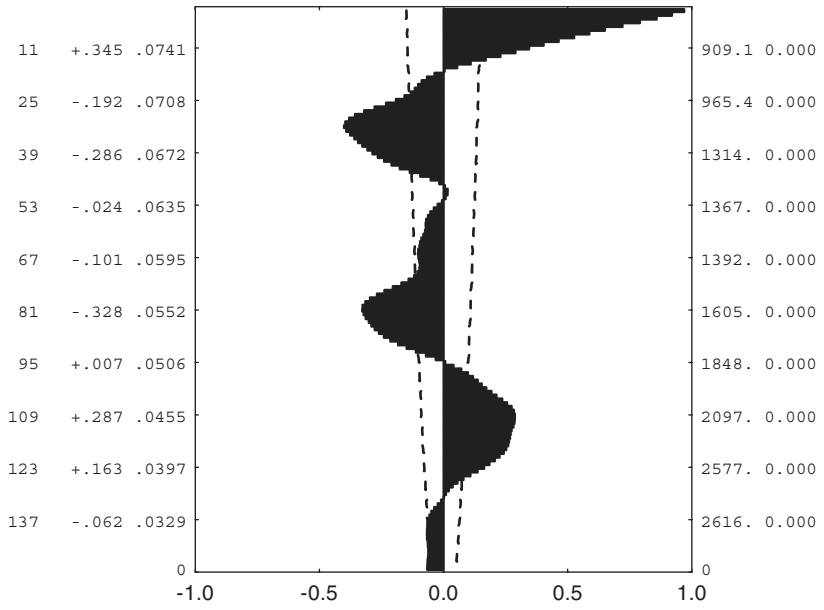


FIG. 5 Autocorrelation plots for hourly temperatures shown in Fig. 4. (A) Lambert's Bay sea-level data, (B) Sneeukop close to Lambert's Bay, (C) Marion Island sea level, (D) Marion Island 800-m site. The dashed lines on each figure represent the 95% confidence intervals, while the values reported to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

(C)



(D)

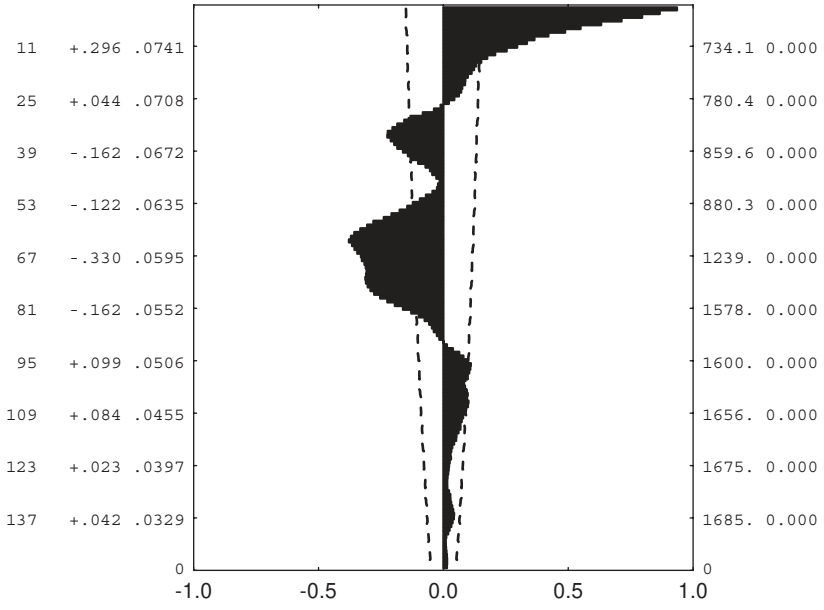


FIG. 5 (continued)

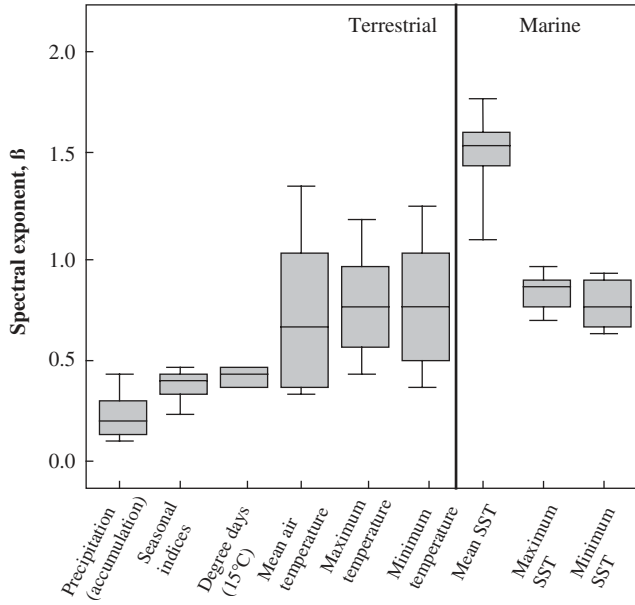


FIG. 6 Box plots of the spectral components for several environmental variables, including sea surface temperature (SST) for terrestrial and marine systems. Lines indicate the median, 75th and 90th percentiles. Redrawn from Vasseur and Yodzis (2004, p. 1149) with permission from the Ecological Society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

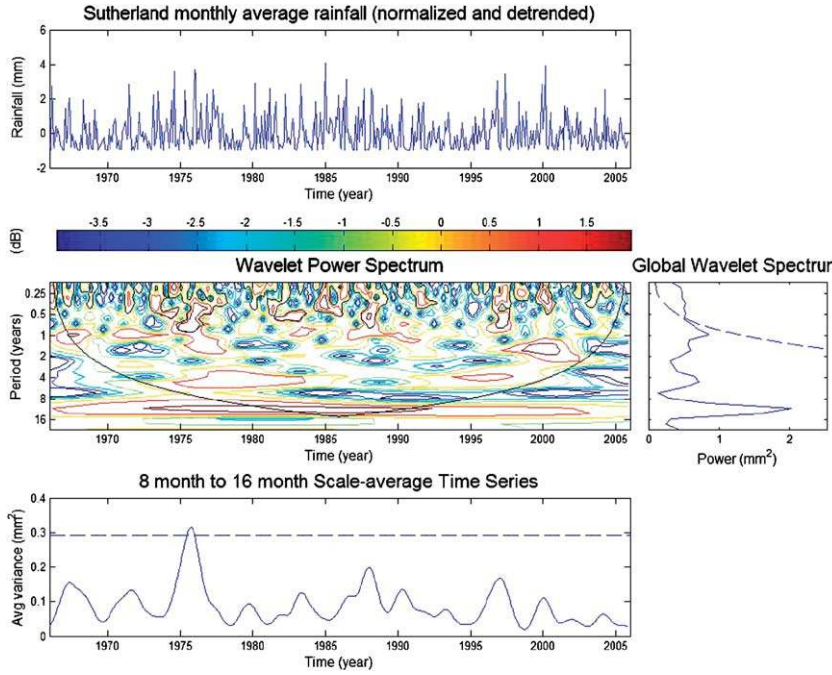


FIG. 7 Wavelet analysis (see Torrence and Compo, 1998) of monthly rainfall data from Sutherland, a high altitude, semi-arid area in the Karoo of South Africa. The upper panel shows the detrended normalized data. The central panel, the wavelet power spectrum with period on the *y*-axis and years on the *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

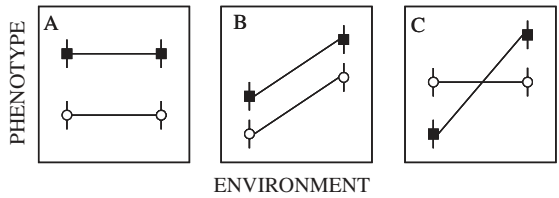


FIG. 8 Reaction norms for two families (circles and squares, mean and standard errors for the phenotypes are shown) demonstrating (A) significant genetic variance, (B) significant genetic and environmental variance, (C) significant genetic, environmental, and genetic by environmental interaction variance.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

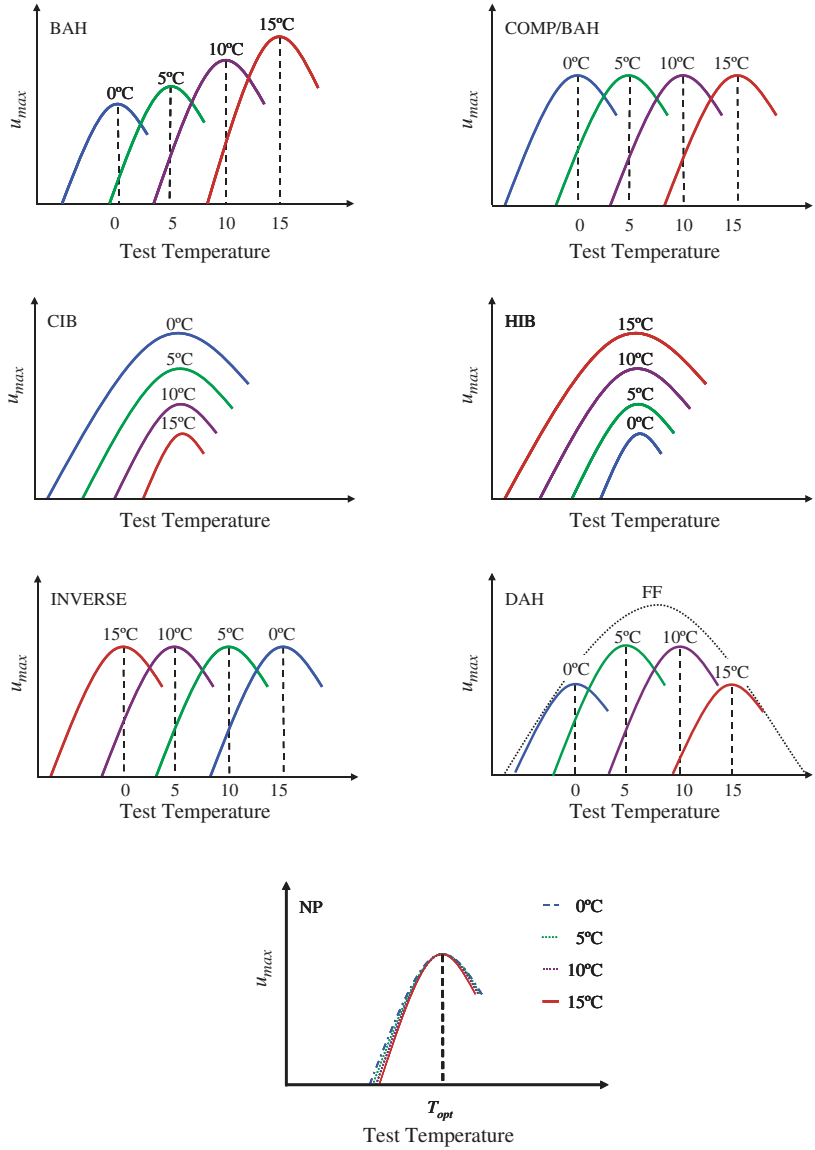


FIG. 9 Predictions from each of the major hypotheses for the response of individual performance curves to acclimation. In each case four acclimation temperatures from low to high are indicated (blue (0 °C), green (5 °C), purple (10 °C), red (15 °C)), and in one case the expectation for field fresh (FF) 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 μl $CO_2\,h^{-1}$, 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

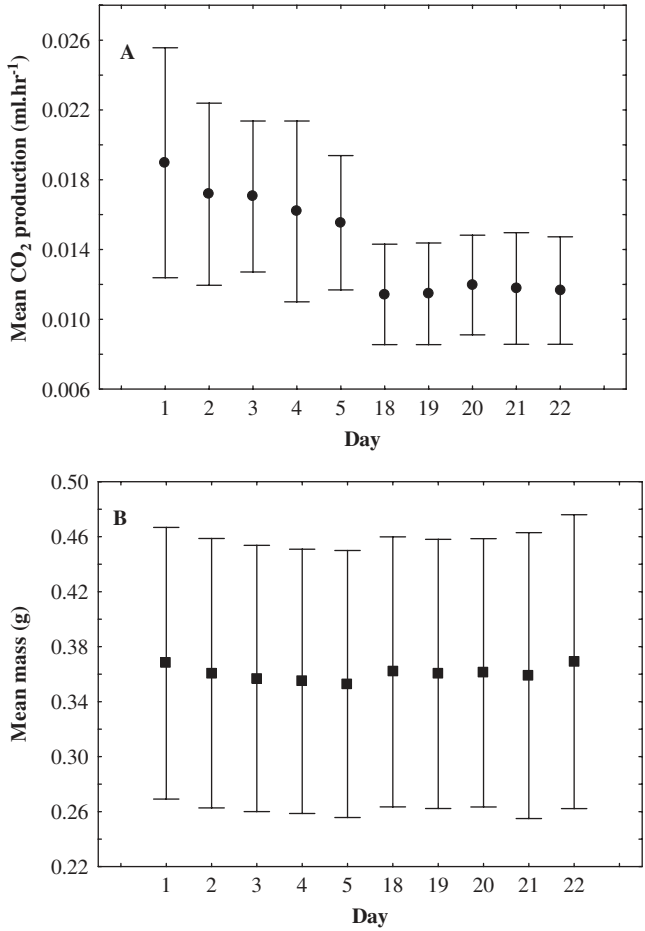


FIG. 10 A rapid decline is found in whole-animal metabolic rate (A) but not in body mass (B) with introduction to stable laboratory conditions in the scorpion *Uroplectes carinatus*. Mean standard metabolic rate (CO_2 ml h^{-1} ± 95% confidence intervals) recorded using flow-through respirometry at 25 °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)

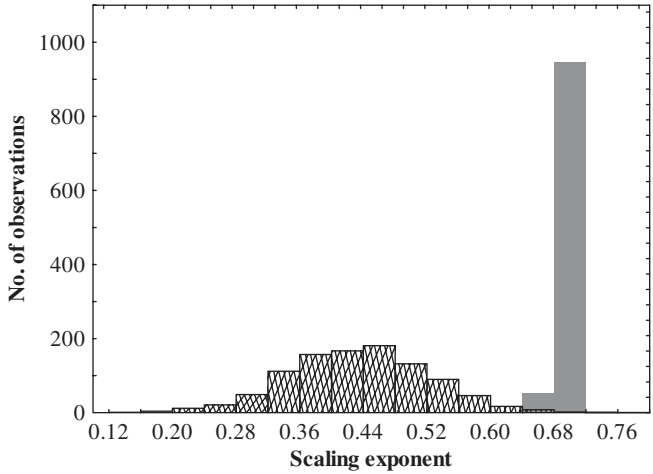


FIG. 11 Variation induced by laboratory acclimation around a hypothetical metabolic rate-body mass scaling relationship. The solid bars represent a hypothetical metabolic rate-body mass scaling relationship for animals ($n = 50$ individuals, using 1000 random numbers re-sampled with replacement using 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

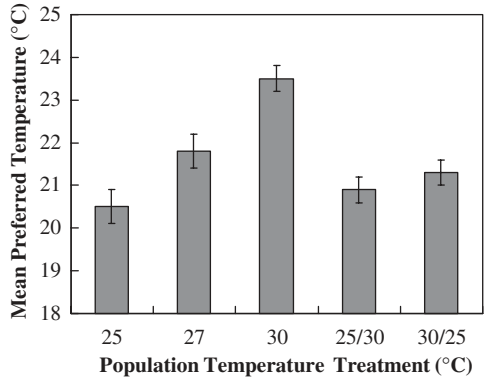


FIG. 12 Mean preferred temperatures (± SE) in *Drosophila melanogaster*. Values represent tenth generation 25, 27, and 30°C-reared populations and reverse temperature treatment populations (i.e. reversible plastic component), 25/30 °C and 30/25 °C (females only). The 25, 27, and 30 °C groups are significantly 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,

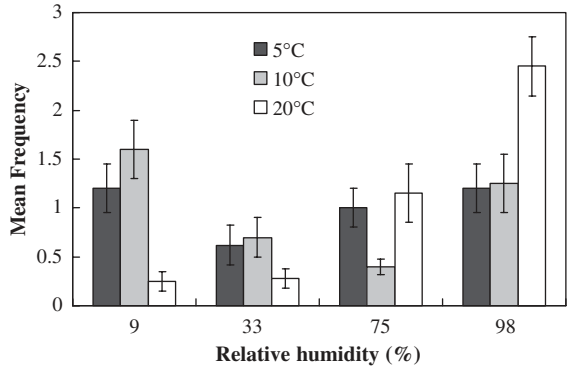


FIG. 13 Mean frequencies (± SE) indicating the distribution of *Cryptopygus antarcticus* within a linear humidity gradient at 5, 10, and 20 °C. These data show that at higher temperatures *C. antarcticus* prefers higher relative humidity. Figure redrawn from Hayward *et al.* (2001) with permission from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

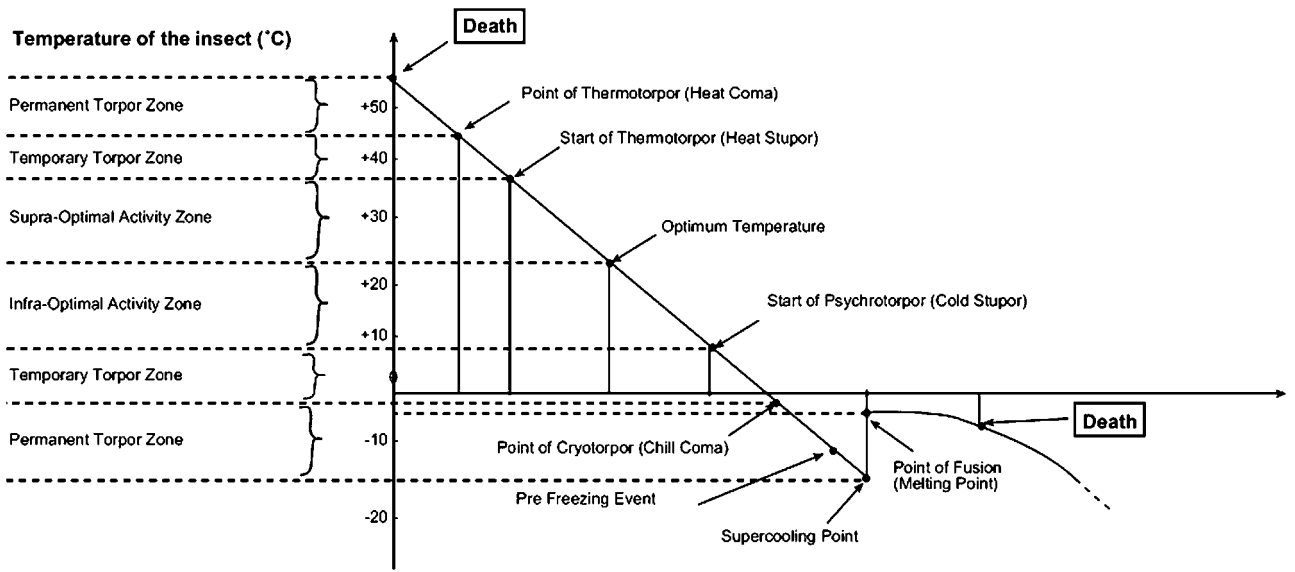


FIG. 14 The thermobiological scale proposed by Vannier (1994). Redrawn with permission from Elsevier.

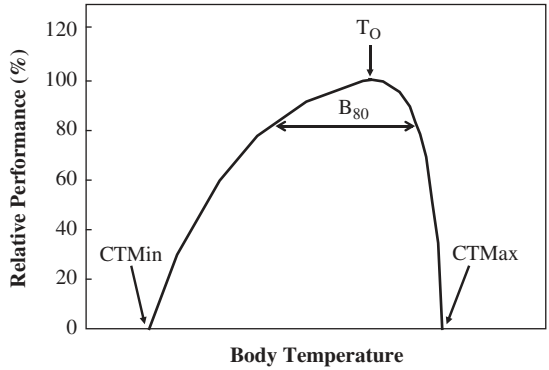


FIG. 15 An idealized thermal performance curve showing the optimum(T_o), performance breadth(B_{80}), and critical limits. Redrawn from Angilletta *et al.* (2002, p. 250) with permission from Elsevier.

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 2×10^3) (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, Ψ, 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 θ 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 θ. 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 θ, 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 θ 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

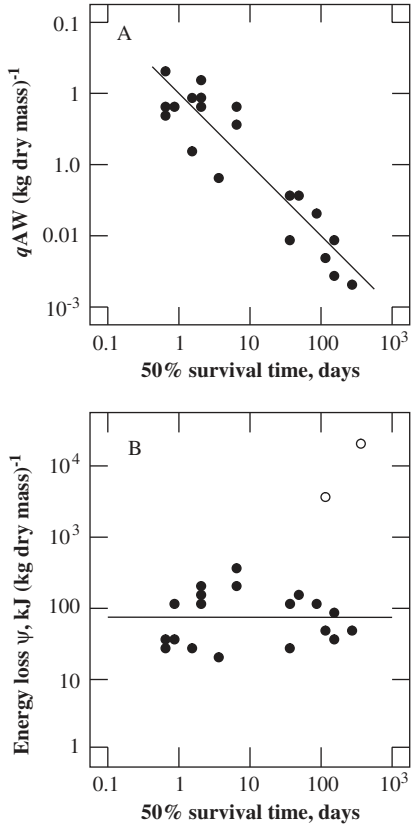


FIG. 16 Metabolic rate, energy loss, and survival time under anoxic conditions. (A) Mass-specific rates, qA, of energy dissipation by organisms capable of surviving more than half a day of anoxia. (B) Energy loss ψ during anoxia is independent of survival time (filled circles). The open circles indicate 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

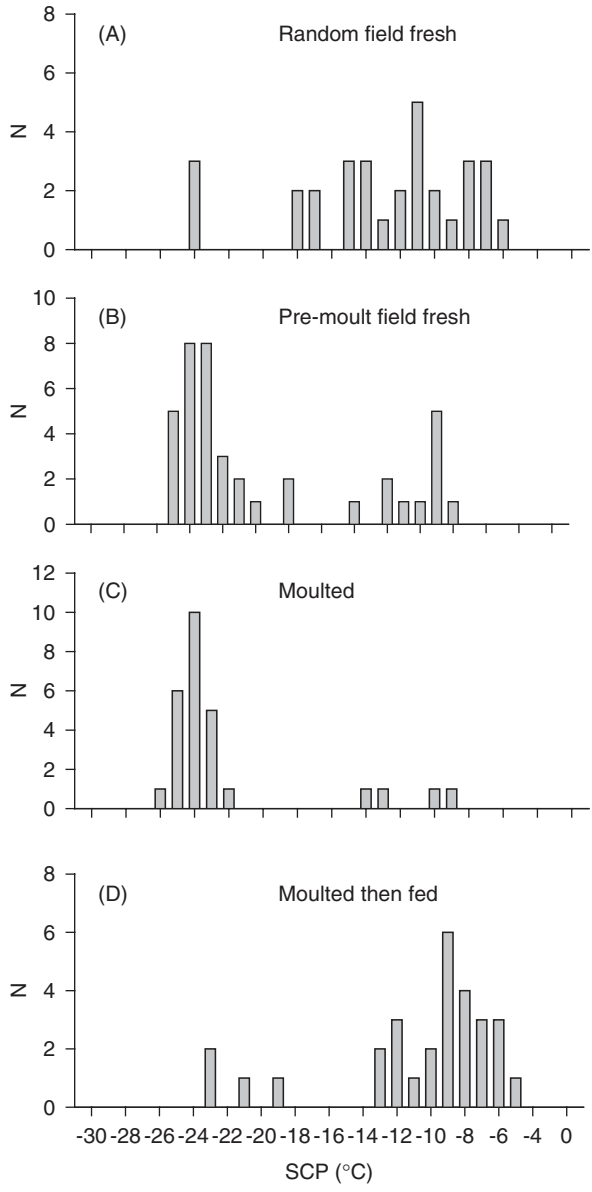


FIG. 17 Supercooling point (SCP) distributions from the springtail *Ceratophysella denticulata* on sub-Antarctic Marion Island from (A) an 'arbitrary' field sample, (B) pre-moulting animals from the same main sample, (C) recently moulted animals, and (D) recently moulted animals that had been fed for 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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