Chapter 1

General introduction

Physiological variation, notably between populations, or between species occupying different environments has long been regarded as evidence of physiological evolution (Garland & Adolph, 1991; Mangum & Hochachka, 1998). In insects, such studies have extended to, *inter alia*, interspecific and interpopulation differences in cold hardiness (e.g. Chen *et al.*, 1990), desiccation resistance or tolerance (e.g. Chown, 1993; Le Legadec *et al.*, 1998), upper lethal temperature limits (e.g. Roberts *et al.*, 1991; Kimura *et al.*, 1994), and metabolic rate (e.g. McKay, 1982; Schultz *et al.*, 1992). In many cases the authors have argued that regular variation in these traits supports the idea of physiological adaptation and evolution. Despite the success of this approach, it is clear that most of the studies on insect physiological ecology have concentrated on variation between a few closely related species or populations (see also Spicer & Gaston, 1999).

However, this is not the only way in which physiological variation might be investigated. Recently, several studies have drawn attention to the fact that large-scale variation in physiological traits is also of considerable interest. For example, Spicer & Gaston (1999) demonstrated that it is only through large-scale investigations of physiological tolerances that issues such as latitudinal variation in thermal tolerances can be addressed. Clearly, such large-scale studies are not novel, but date to the early works on metabolic cold adaptation (e.g. Scholander *et al.*, 1953) and the scaling of metabolic rate (e.g. Rao & Bullock, 1954; Bartholomew & Casey, 1978). However, they are becoming increasingly important as ecology turns to large-scale investigations as a means to understand both local patterns, and those that are evident at continental to global scales (Lawton 1999; Spicer & Gaston, 1999).

In seeking to explain the patterns they document, macroecologists have made a variety of assumptions concerning variation in physiological traits over both large and small spatial scales, and their distribution across species and higher taxa. Three examples serve to illustrate this point. First, in explaining geographic variation in range size, it has been hypothesized that the range of species thermal tolerances increases both with latitude and altitude (Stevens, 1989; Gaston *et al*., 1998). Second, one of the mechanisms proposed to account for higher species richness in the tropics is a rapid rate of speciation in this warm region, associated with multiple generations per year (Rohde, 1992, 1997), which must in turn be a consequence of large scale variation in development rate and number of generations per season. Third, in investigating patterns in the range size of species, the question of the determinants of range edges has been raised repeatedly (Brown, 1995; Gaston & Blackburn, 2000). In many instances it is argued that interactions between abiotic factors and species physiological tolerances are important contributors to species range limits (Brown *et al*., 1996), although the precise mechanics of such range limitation, especially the likelihood of physiological differences between central and marginal populations, remain a subject of vigorous discussion (Hoffmann & Blows, 1994; Kirkpatrick & Barton, 1997; Lennon *et al*., 1997; Chown & Gaston, 1999; Hofmann & Hewa-Kapuge, 2000; Holt & Keitt, 2000). The latter question is gaining in importance as humans alter global climates, and these in turn precipitate large changes to animal and plant species and communities (Kareiva *et al*., 1993; Parmesan *et al*., 1999; van Jaarsveld & Chown, 2001).

At virtually the same time, physiologists and ecologists have realized that comparative studies can no longer rely on straightforward statistical techniques, especially where species are closely related. That is, species cannot always be regarded as independent data points in analysis owing to phylogenetic non-independence of the data (Harvey & Pagel, 1991; Garland & Adolph, 1994). Thus, many recent studies have emphasised the importance of phylogenetics in comparative biology and have also suggested that older conclusions may have to be revisited (e.g. Read & Harvey, 1989; Gaston, 1998; Magnum & Hochachka, 1998; Chown, 2001). However, the need both for revisiting older conclusions, and incorporating phylogenetic analysis in future studies does depend on how physiological variation is partitioned between taxonomic levels. If physiological variation is partitioned at higher taxonomic levels, then a comparative method incorporating phylogenetic information would give more reliable results (see Harvey, 1996).

In short, it appears that there is an increasing demand for investigations of large-scale variation in physiological traits, both spatially, and in terms of their taxonomic variation (see also Spicer & Gaston, 1999). It is the primary aim of this study therefore to provide a quantitative analysis of several insect ecophysiological parameters to address this demand. In essence, the work presented here will determine whether within each of the fields selected the geographic extent of the studies has been sufficiently comprehensive for large-scale variation to be detected, and if this is the case, what form this variation takes. This study will also consider the extent to which variance in the physiological variables selected is partitioned at higher taxonomic levels, and in some cases, how these parameters vary with body size. In so doing, the study seeks to provide a fresh look at what we know about the role of ecophysiological tolerance in determining macroecological patterns in insects, and to encourage the development of an interaction between macroecology and physiological ecology across all major taxa.

To this end, five major variables have been selected for analysis, i.e. upper and lower lethal temperatures, water loss rate, rate of development, and metabolic rate. The rationale for the selection of these variables is as follows:

- One of the major hypotheses put forward to explain the positive relationship between latitudinal/altitudinal extent and latitude/altitude (i.e. Rapoport's Rule, see Stevens, 1989) is the increase in physiological tolerance range that more temperate species have compared to those from tropical regions. In other words, tropical species should have narrower tolerance limits than those from polar regions (Gaston *et al.*, 1998). At least one study has demonstrated such a pattern over an altitudinal gradient (Gaston & Chown, 1999), but this has not been done for latitude. For this reason, upper and lower lethal temperatures were selected. These parameters have also been widely examined in insects.

- Water is one of the key climatic factors in terrestrial ecosystems that is thought to influence the distribution and abundance of plants and animals (Vollmer & MacMahon, 1974). Many studies have emphasized the relationship between the rate of water loss of organisms and their habitat (see Cloudsley-Thompson, 1961, 1967; Ahearn, 1970), though few studies have sought to test this relationship at broad spatial scales. In addition, studies of dry habitat beetle species have suggested that there is a strong causal relationship between metabolic rate and total water loss, because in these species cuticular transpiration has been reduced to a minimum (Zacharissen *et al.*, 1987; Zacharissen *et al.*, 1988). However, many other studies have suggested that the contribution of respiratory transpiration to total water loss is so small, that any alterations to metabolic rate are unlikely to influence overall water loss (for review see Bosch *et al.*, 2000). Clearly, the relationship between metabolic rate and water loss deserves further exploration using more species and covering a wider geographical area. For these reasons water loss rate was investigated.

- To explain the latitudinal gradient in species richness (Gaston, 1996; Gaston & Blackburn, 2000), Rohde (1992) argued that the rate of evolutionary change is greater at low latitudes than at high latitudes. He argued that this greater evolutionary rate in the tropics is due to factors such as shorter generation time of many low latitude species, an increase in the rates of mutation resulting from higher tropical temperatures, and acceleration of selection leading to fixation of favourable mutants in populations. In insects at least, the question of shorter generation times at low latitudes has not been addressed in this context. Indeed there have been very few tests of Rohde's (1992) ideas. For this reason, development rate was selected as a variable of interest. Although there has been much recent work on insect development rates by Honêk and colleagues (see e.g. Honêk & Kocourek, 1990; Honêk 1996), they sought to address a somewhat different set of issues. Here, the work of Honêk is extended to address the issue of the likelihood of extended generation times at high latitudes. This dataset is also used to examine the evidence for compensation in development time in temperate vs tropical species (for discussion see Clarke, 1993, Trudgill, 1995; Chown & Gaston, 1999).

- Finally, metabolic rate was investigated not only because it has formed an important component of recent models of body size evolution (Brown *et al.*, 1993; Chown & Gaston, 1997), but also because latitudinal variation in this parameter is likely to have some influence on variation in species energy use across latitudes (Currie & Fritz, 1993). How species energy use varies between animals of different body sizes and with latitude are polemical, though important, macroecological questions because they touch on how assemblages are likely to be structured (Gaston & Blackburn, 2000), and on how energy abstraction from the environment by humans might alter animal assemblages. Thus, variation in metabolic rate in species from different latitudes is investigated to determine whether metabolic cold adaptation is present in insects over large scales (for additional discussion see Bullock, 1955; Cossins & Bowler, 1987; Aunaas *et al.*, 1983; Stromme *et al.*, 1986; Schultz *et al.*, 1992, Crafford & Chown, 1993, Todd, 1997; Chappell, 1983; Massion, 1983; Block, 1977; Block & Young, 1978; Young, 1979; Chown & Gaston, 1999), and whether this also extends to latitudinal variation in the slopes of metabolic rate temperature curves.

References

Ahearn, G.A. 1970. The control of water loss in desert tenebrionid beetles. *Journal of Experimental Biology* **53**, 573-595.

Aunaas, T., Baust, J.G. & Zachariassen, K.E. 1983. Ecological studies on arthropods from Spitbergen. *Polar Research* **1**, 235-240.

Bartholomew, G.A. & Casey, T.M. 1978. Oxygen consumption of moths during rest, pre-flight warm-up, and flight in relation to body size and wing morphology. *Journal of Experimental Biology* **76**, 11-25.

Block, W. 1977. Oxygen consumption of the terrestrial mite *Alaskozetes antarcticus* (Acari: Cryptostigma). *Journal of Experimental Biology* **68**, 69-87.

Block, W. Young, S.R. 1978. Metabolic adaptations of Antarctic terrestrial micro-arthropods. *Comparative Biochemistry and Physiology* **61A**, 363-368.

Bosch, M., Chown, S.L. & Scholtz, C.H. 2000. Discontinuous gas exchange and water loss in the keratin beetle *Omorgus radula*: further evidence against the water conservation hypothesis? *Physiological Entomology* **24**, 309-314.

Brown, J.H. 1995. *Macroecology*. University of Chicago Press, Chicago.

Brown, J.H., Marquet, P.A. & Taper, M.L. 1993. Evolution of body size: consequences of an energetic definition of fitness. *American Naturalist* **142**, 573-584.

Brown, J.H., Stevens, G.C., Kaufman, D.M. 1996. The geographic range: size, shape, boundaries, and internal structure. *Annual Review of Ecology and Systematics* **27**, 597-623.

Bullock, T.H. 1955. Compensation for temperature in the metabolism and activity of poikilotherms. *Biological Reviews* **30**, 311-342.

Chapell, M.A. 1983. Metabolism and thermoregulation in desert and montane grasshoppers. *Oecologia* **56**, 126-131.

Chen, C.P., Lee, R.E. & Delinger, D.L. 1990. A comparison of the responses of tropical and temperate flies (Diptera: Sarcophagidae) to cold and heat stress. *Journal of Comparative Physiology B* **160**, 543-547.

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

Chown, S.L. 2001. Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* (in press).

Chown, S.L. & Gaston, K.J. 1997. The species-body size diatribution: energy, fitness and optimality. *Functional Ecology* **11**, 365-375.

Chown, S.L. & Gaston, K.J. 1999. Exploring the links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews* **74**, 87-120.

Clarke, A. 1993. Seasonal acclimation and latitudinal compensation in metabolism: do they exist? *Functional Ecology* **7**, 139-149.

Cloudsley,-Thompson, J.L. 1961. Observations on the natural history of the 'camel spider' *Galeodes arabs* (Solifugae: Galeodidae) in the Sudan. *Entomologist's Monthly Magazine* **98**, 243-246.

Cloudsley,-Thompson, J.L. 1967. The water relations of scorpions and tarantulas from the Sonoran desert. *Entomologist's Monthly Magazine* **103**, 217-220.

Cossins, A.R. & Bowler, K. 1987. *Temperature Biology of Animals*. Chapman & Hall, London.

Crafford, J.E. & Chown, S.L. 1993. Respiratory metabolism of sub-Antarctic insects from different habitats on Marion Island. *Polar Biology* **13**, 411-415.

Currie, D.J. & Fritz, J.T. 1993. Global patterns of animal abundance and species energy use. *Oikos* **67**, 56-68.

Garland, T. & Adolph, S.C. 1994. Why not do two-species comparative studies: limitations on inferring adaptation. *Physiological Zoology* **67**, 797-828.

Gaston, K.J. 1996. Biodiversity-latitudinal gradients. *Progress in Physical Geography* **20**, 466-476.

Gaston, K.J. 1998. Species-range size distributions: products of speciation, extinction and transformation. *Philosophical Transactions of the Royal Society of America* **353B**, 219-230.

Gaston, K.J. & Blackburn, T.M. 2000. *Patterns and Progress in Macroecology*. Blackwell Science, Oxford.

Gaston, K.J., Blackburn, T.M. & Spicer, J.I. 1998. Rapoport's rule: time for an epitaph? *Trends in Ecology & Evolution* **13**, 70-74.

Gaston, K.J. & Chown, S.L. 1999a. Why Rapoports rule does not generalise. *Oikos* **84**, 309-312.

Gaston, K.J. & Chown, S.L. 1999b. Elevation and climatic tolerance: a test using dung beetles. *Oikos* **86**, 584-590.

Harvey, P.H. 1996. Phylogenies for ecologists. *Journal of Animal Ecology* **65**, 225-263.

Harvey, P.H. & Pagel, M.D. 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.

Hoffmann, A.A. & Blows, M.W. 1994. Species borders: ecological and evolutionary perspectives. *Trends in Ecology & Evolution* **9**, 223-227.

Hoffmann, A.A. & Hewa-Kapuge, S. 2000. Acclimation for heat resistance in *Trichogramma sp. nr. brassicae*: can it occur without costs? *Functional Ecology* **14**, 55-60.

Holt, R.D. & Keitt, 2000. Alternative causes for range limits: a metapopulation perspective. *Ecology Letters* **3**, 41-47.

Honêk, A. 1996. Geographical variation in thermal requirements for insect development. *European Journal of Entomology* **93**, 303-312.

Honêk, A. & Kocourek, F. 1990. Temperature and development time in insects: a general relationship between thermal constants. *Zoologische Jahrbuecher Systematik* **117**, 401-439.

Johnston, .A., Calvo, J., Guderley, H., Fernandez, D. & Palmer, L. 1998. Latitudinal variation in the abundance and oxidative capabilities of muscle mitochondria in perciform fishes. *Journal of Experimental Biology* **201**, 1-12.

Kareiva, P.M., Kingsolver, J.G. & Huey, R.B. 1993. *Biotic Interactions and Global Change*. Sinauer Associates, Sunderland.

Kimura, M.T., Ohtsu, T., Yoshida, T. Awasaki, T. & Lin F-J. 1994. Climatic adaptations and distributions in the *Drosophila takahashii* species subgroup (Diptera: Drosophilidae). *Journal of Natural History* **28**, 401-409.

Kirkpatrick, M. & Barton, N.H. 1997. Evolution of species' range. *American Naturalist* **150**, 1-23.

Lawton, J.H. 1999. Are there general laws in ecology? *Oikos* **84**, 177-192.

Le Legadec, M.D., Chown, S.L. & Scholtz, C.H. 1998. Desiccation resistance and water balance in southern African keratin beetles (Coleoptera: Trogidae): the influence of body size, habitat and phylogeny. *Journal of Comparative Physiology B* **168**, 112-122.

Lennon, J.J., Turner, J.R.G. & Connell, D. 1997. A metapopulation model of species boundaries. *Oikos* **78**, 486-502.

MacKay, W.P. 1982. An altitudinal comparison of oxygen consumption rates in three species of *Pogonomyrmex harvester* ants (Hymenoptera: Formicidae). *Physiological Zoology* **55**, 367-377.

Mangum, C.P. & Hochachka, P.W. 1998. New directions in comparative physiology and biochemistry: mechanisms, adaptations, and evolution. *Physiological Zoology* **71**, 471-484.

Massion, D.D. 1983. An altitudinal comparison of water loss and metabolism relations in two acridid grasshoppers (Orthoptera). *Comparative Biochemistry and Physiology* **74A**, 101-105.

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J.K., Thomas, C.D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W.J., Thomas, J.A. & Warren, M. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **399**, 579-583.

Rao, K.P. & Bullock, T.H. 1954. Q_{10} as a function of size and habitat temperature in poikilotherms. *American Naturalist* **88**, 33-44.

Read, A.F. & Harvey, P.H. 1989. Life history differences among the Eutherian rations . *Journal of Zoology, London* **219**, 329-353.

Roberts, C.S., Seely, M.K., Ward, D., Mitchell, D. & Campbell, J.D. 1991. Body temperatures of Namib desert tenebrionid beetles: their relationship in laboratory and field. *Physiological Entomology* **16**, 463-475.

Rohde, K. 1992. Latitudinal gradients in species diversity: the search for the primary cause. *Oikos* **65**, 514-527.

Rohde, K. 1997. The larger area of the tropics does not explain latitudinal gradients in species diversity. *Oikos* **79**, 169-172.

Scholander, P.F., Flagg, W., Walters, V. & Irving, L. 1953. Climatic adaptations in arctic and tropical poikilotherms. *Physiological Zoology* **26**, 67-92.

Schultz, T.D., Quinlan, M.C. & Hadley, N.F. 1992. Preferred body temperature, metabolic physiology, and water balance of adult *Cicindela longilabris*: a comparison of populations from aboreal habitats and climatic refugia. *Physiological Zoology* **65**, 226-242.

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

Stevens, G.C. 1989. The latitudinal gradient in geographic range: how so many species coexist in the tropics. *American Naturalist* **133**, 240-256.

Stromme, J.A., Ngari, T. & Zachariassen, K.E. 1986. Physiological adaptations of coleoptera on Spitsbergen. *Polar Research* **4**, 199-204.

Todd, C.M. 1997. Respiratory metabolism in two species of carabid beetles from the sub-Antarctic Island of South Georgia. *Polar Biology* **18**, 166-171.

Trudgill, D.L. 1995. Why do tropical poikilothermic organisms tend to have higher threshold temperatures for development than temperate ones? *Functional Ecology* **9**, 136-137.

Van Jaarsveld, A.S. & Chown, S.L. 2001. Climate change and the impacts in South Africa. *Trends in Ecology & Evolution* **16**, 13-14.

Vollmer, A.T. & MacMahon, J.A. 1974. Comparative water relations of five species of spiders from different habitats. *Comparative Biochemistry and Physiology* **47A**, 753-765.

Young, S.R. 1979. Respiratory metabolism of *Alaskozetes antarcticus*. *Journal of Insect Physiology* **25**, 361-369.

Zachariassen, K.E. 1985. Physiology of cold tolerance in insects. *Physiological Review* **65**, 799-832.

Zachariassen, K.E., Andersen, J., Maloiy, G.M.O. & Kimau, J.M.Z. 1987. Transpiratory water loss and metabolism of beetles from arid areas in East Africa. *Comparative Biochemistry and Physiology* **86A**, 403-408.

Zachariassen, K.E., Andersen, J., Kimau, J.M.Z. & Maloiy, G.M.O. 1988. Water loss in insects from arid and humid habitats in East Africa. *Acta Entomologica Bohemoslovaca* **85**, 81-93.

Chapter 2

Thermal tolerance, climatic variability and latitude

Published in *Proceedings of the Royal Society of London B* **267**, 739-745 (2000)

2.1 Introduction

Rapoport's rule, the increase in the latitudinal extents of occurrence of species towards higher latitudes, has attracted increasing interest recently (Roy *et al.*, 1994, 1998; Blackburn & Gaston, 1996; Rohde, 1996; Fleishman *et al.*, 1998; Gaston *et al.*, 1998; Johnson 1998; Ruggiero & Lawton, 1998; Santelices & Marquet, 1998; Gaston & Chown, 1999a). According to Stevens (1989), the mechanism largely responsible for producing this macroecological pattern is straightforward. To survive at higher latitudes individual organisms need to be able to withstand greater temporal variability in climatic conditions than at lower latitudes. In consequence, the species to which these individuals belong can attain wider latitudinal extents at higher latitudes. This climatic variability hypothesis has a distinguished history (see Gaston & Chown, 1999a for review), and Stevens (1989) argues that it actually applies to the distributions of species across most environmental gradients (e.g. Stevens, 1992, but see Rohde, 1992; Roy *et al.*, 1998).

Two assumptions are critical to the climatic variability hypothesis. It requires first that there is indeed an appropriate gradient (latitudinal, altitudinal, or otherwise) in climatic variability, and second, that there is a matching cline in the physiological tolerances of species, probably most significantly in thermal tolerances (Gaston *et al.*, 1998). Although there is substantial evidence in support of the former assumption (Stevens, 1989, 1992; Gaston & Chown, 1999a), the latter has seldom been assessed (Gaston *et al.*, 1998; Spicer & Gaston, 1999). The only study that explicitly set out to investigate both assumptions, for dung beetles on an altitudinal gradient, concluded that there was some support for both (Gaston & Chown, 1999b). The authors also demonstrated that variation in the thermal tolerances of the species concerned was due more to a cline in lower than upper thermal limits, and argued that this may be true for latitudinal clines too (see also Lutterschmidt & Hutchinson, 1997; Goto & Kimura, 1998).

Despite the obvious importance of so doing, variation in the thermal tolerances of species across a large latitudinal gradient has to date been little investigated (see Scholander *et al.*, 1953 for pioneering attempts, and Spicer & Gaston, 1999 for further review). Clearly, a field investigation such as that undertaken by Gaston & Chown (1999b) could be performed at larger scales to address this problem. However, synthetic analyses of available physiological information provide an alternative first step.

Here the results of a synthetic, quantitative analysis of latitudinal patterns of variation in insect cold hardiness or lower lethal temperature limits (both lower lethal temperature (LLT), and crystallization temperature or supercooling point (SCP), see Block, 1990; Lee & Denlinger, 1991; Bale, 1993; Sømme, 1999), and insect upper thermal limits (both critical thermal maxima (CTMax), and upper lethal temperatures (ULT), see Lutterschmidt & Hutchinson, 1997) based on the published literature. To determine the extent of such variation for both upper and lower lethal limits and whether this provides support for the physiological tolerance assumption of the climatic variability hypothesis. In addition to (i) determine whether the classification scheme presently used for patterns in insect cold hardiness (Block, 1990; Bale, 1993; Sinclair, 1999; Sømme, 1999) has substantial quantitative support, because the outcome of such an analysis would, to a large extent, determine the approach adopted in all subsequent analyses; (ii) examine variation in cold hardiness strategy (freezing tolerant or intolerant, see below) between life stages because this determines whether all stages can be included in the broad scale analyses of lower lethal limits (the bulk of the upper lethal limit data are for adult insects); and (iii) determine the partitioning of variation in LLT, SCP, CTMax, and ULT amongst taxonomic levels, because such partitioning of physiological variation has been poorly documented in animal groups (Spicer & Gaston, 1999), and again has implications for the interpretation of other results.

2.2 Materials and methods

The study of the upper and lower lethal limits was chosen in insects mostly because these limits have been studied in many taxa, and because the physiological principles underlying thermal tolerance have been comprehensively assessed at several levels of organization. Insect lower lethal limits have been widely investigated over the past 50 years (Sømme, 1982, 1999; Block, 1990; Duman *et al.*, 1991; Lee & Denlinger, 1991; Danks, 1996; Sinclair, 1999), and their physiological basis is well known (Zachariassen, 1985; Storey & Storey, 1996; Sømme, 1999). The same is generally true of insect upper lethal limits (e.g. Vannier, 1994; Lutterschmidt & Hutchinson, 1997), although a full appreciation of the importance of heat shock proteins in promoting tolerance of heat stress in insects is more recent (see Feder & Hofmann, 1999 for review)

The relevant literature on insect cold hardiness, excluding a number of Russian studies that could not readily be obtained, was assessed as far back as 1900 (1928-1998), but with major emphasis placed on the last 50 years. Over 400 published papers were examined. The following studies were excluded from the analyses: (i) Those dealing with insects treated with bacteria or liquid nitrogen, and those where insects were pierced with the thermocouples used for the investigation, because SCPs and LLTs are altered by such treatments (Sømme, 1982; Lee *et al.*, 1993); and (ii) Studies of insects collected in their summer activity period and those using insects from laboratory colonies. It has been shown that summer acclimatised (or acclimated) insects are generally not cold hardy (Block, 1990; Lee & Denlinger, 1991; Storey & Storey, 1996; Sømme, 1999). Following this selection a second, iterative protocol was employed. Where a species was examined more than once, either in a single paper or by different authors, the record with the lowest SCP was selected and the other records were set aside. If a species was encountered twice or more with the same SCP, then the record from the geographic location that was least represented in the database was selected. However, if different development stages had been investigated, data from each of these stages were included, but assessed in the same manner. Thus data on 250 species belonging to 87 families and ten orders were extracted from a total of 175 published papers.

From each of these studies the following information was obtained: SCP, LLT, geographic location, season, stage of development, and whether the authors considered the insect to be freezing tolerant or freezing intolerant. Until fairly recently, most cold hardy insects were classified as either able to tolerate freezing of water in their extracellular spaces (freezing tolerant), or as unable to do so (freezing intolerant) (Block, 1990; Lee & Denlinger, 1991; Storey & Storey, 1996; but see Bale, 1996). In those studies where the LLT was not reported, only the SCP was used. No assumption of equivalence of SCP and LLT was made (see Bale, 1993, 1996 for rationale). For the purpose of analysis the mean SCP and/or LLT reported in a study were used because in some cases authors provided only means, while others included a measure of variation (e.g. range, standard deviation). This assumes that both SCP and LLT show a normal distribution of values, a reasonable assumption for winter acclimatised individuals (Klok & Chown, 1998).

Two major methods for determining upper thermal tolerance are generally employed. For the static or upper lethal temperature (ULT) method the time to death at constant test temperatures is determined. The dynamic method, often known as the Critical Thermal Maximum (CTMax), involves increasing the test temperatures until an end point, often the onset of spasms, is reached. 272 papers, which employed either or both methods, were examined (1900 onwards, see above). From each of these, the following information was extracted: ULT and/or CTMax, geographic location, season, and stage of development. Data were not used from studies where an insect was exposed to high temperatures for more than 24 h because death due to starvation or other factors could not be ruled out. Likewise, data from treatments including modified atmospheres were also discarded. An iterative protocol, similar to that described above, was then employed. Where a species was examined more than once, either in a single paper or by different authors, the record with the highest ULT or CTMax was selected. If a species was entered twice or more with the same ULT or CTMax, then the record from the geographic location that was least represented in the database was selected. Because data from adult insects predominated (>70%) we excluded immature stages from the analysis (see above). Thus data from a total of 127 published papers were used.

2.3 Results and discussion

Inspection of the cold hardiness database revealed that where cold hardiness in all or most stages of a given species has been investigated (n = 16, freezing tolerant = 2, freezing intolerant = 14), the strategy (freezing tolerant or intolerant) generally does not differ between them (94% of the species showed no difference). Thus we did not distinguish between developmental stage in initial assessments of the data. All stages of a given species were also included if more than one stage was investigated, because SCPs vary significantly with stage, at least in the freezing intolerant species (SCP freezing intolerant species: ANOVA $F_{3,\,204} = 30.53$, $p < 0.00001$; SCP freezing tolerant species: ANOVA $F_{1,\,129} = 0.008$, $p > 0.9$; LLT freezing tolerant species: ANOVA $F_{1,\,98} = 0.345$, $p > 0.5$). However, to avoid problems associated with statistical non-independence, all statistical analyses were undertaken using data for adult insects whenever data for more than one stage were available, but data for the other stages when only these were available (if both larval and pupal values were available, only one of these was used based on the iterative protocol). This was done because the majority of the studies used concerned adult insects.

A bivariate scatterplot of SCP and LLT, including a line of equivalence of the two (see Sinclair, 1999 for rationale), was used in conjunction with the conclusions of the authors who undertook a particular investigation, to determine the extent to which species could be assigned to one of the two classical cold hardiness strategies. Freezing intolerant species are generally well differentiated from freezing tolerant ones (Fig. 2.1). Most species originally identified as freezing intolerant have equivalent SCPs and LLTs (Fig. 2.1). Nonetheless, within each of the categories there is clearly considerable variation, including some instances of pre-freeze mortality in freezing intolerant species, and a wide range of SCPs and LLTs in freezing tolerant species (Fig. 2.1). Such variation has been widely discussed (Ring, 1982; Bale, 1993; Klok & Chown, 1997; Sømme, 1999; Sinclair,

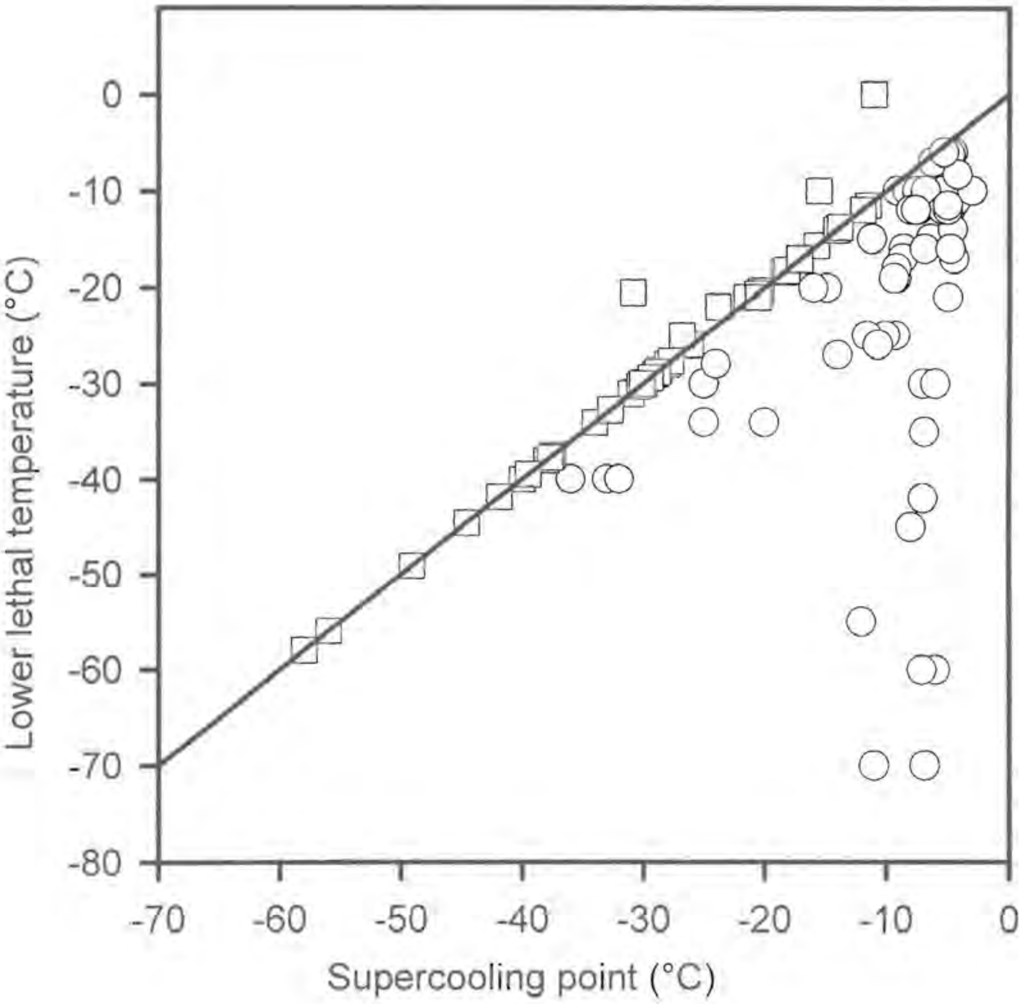

Figure 2.1. Plot of lower lethal temperature (LLT) against supercooling point (SCP) of both freezing tolerant (○) and freezing intolerant insect species (□). The line of equivalence is also shown.

1999), and it encompasses that shown by a small number of species known to survive only partial extracellular ice formation (e.g. Sinclair *et al.*, 1999), and at least one species that is known to have switched from being freezing intolerant to freezing tolerant over a period of a year (Kukal & Duman, 1989). The importance of pre-freeze mortality in insects has been discussed by a variety of authors (e.g. Knight *et al.*, 1986), and forms the basis of a somewhat polemical, though useful extended cold hardiness classification (Bale, 1987, 1993, 1996; Sømme, 1999). This analysis suggests that while the classical distinction between freezing tolerant and freezing intolerant species may no longer be sufficient for describing the full range of variation in cold hardiness found in insects, it remains useful as a rule distinguishing the strategies generally adopted by insects because the dichotomy is well supported (Lawton, 1999).

To investigate the taxonomic levels at which most variance in cold hardiness was partitioned, SCP and LLT values for the freezing tolerant species, and SCP values for the freezing intolerant ones (due to equivalence of SCP and LLT, Fig. 2.1), were subjected separately to nested analysis of variance using the Satterthwaite approximation for unequal sample sizes. Of the 10 orders investigated, two include freezing tolerant species only (Blattodea 3 species examined; Neuroptera 1 sp.), and two freezing intolerant species only (Mecoptera 2 spp.; Siphonaptera 1 sp.). Both strategies are found in the remaining six orders (Orthoptera 8 spp. examined, Hemiptera 30 spp., Coleoptera 91 spp., Diptera 33 spp., Lepidoptera 42 spp., Hymenoptera 39 spp.). Therefore the current evidence does not support the idea of strong phylogenetic constraint with regard to the evolution of these strategies, nor does it support the notion that freezing tolerance is restricted to holometabolous insects (Block, 1982, 1990). Nonetheless, within each of the strategies there is evidence of reasonably pronounced phylogenetic constraints on the evolution of SCP and LLT. In both the freezing tolerant and intolerant groups, the bulk of the variance in supercooling point was partitioned equally among families and among genera, with somewhat less among orders, and the least among species (Table 2.1).

Table 2.1. Distribution of variance in supercooling point (SCP) of the freezing intolerant and freezing tolerant insect groups, and lower lethal temperature (LLT) of the freezing tolerant group. Tabulated values are percentage of the total variance accounted for at each successive level. The species level includes the error term in the data. (* $p < 0.05$, ** $p < 0.001$)

Trait/level	Species	Genus	Family	Order
Freezing intolerant SCP	15.71	33.14**	32.47*	18.68*
Freezing tolerant SCP	1.85	44.12**	40.85**	13.18
Freezing tolerant LLT	32.81	0.06	46.87**	20.26

Likewise for LLT of the freezing tolerant species the bulk of the variance was partitioned among families, but in this case a considerably greater proportion of the variance was partitioned among species, and much less among genera (Table 2.1). Variance partitioning in upper lethal temperatures was examined using the same methods. CTMax data were available for 6 orders and 24 families, and ULT data for 11 orders and 37 families. Within each of the data sets, there is evidence of phylogenetic constraint similar to that found for cold hardiness. For both CTMax and ULT the bulk of the variance was partitioned either at the family or generic level, with less among species, and the least among orders (Table 2.2). These findings are not unusual for many life history variables (Read & Harvey, 1989; Harvey & Pagel, 1991), although taxonomic variation in physiological traits is less well understood (Chown *et al.*, 1999; Spicer & Gaston, 1999).

Table 2.2. Distribution of variance in critical thermal maxima (CTMax) and upper lethal temperatures (ULT). Tabulated values are percentage of the total variance accounted for at each successive level. The species level includes the error term in the data. (* $p < 0.05$, ** $p < 0.001$)

Trait/level	Species	Genus	Family	Order
CTMax	25.86	57.72**	12.62	3.8
ULT	22.78	29.89**	46.61**	0.72

Variation in SCP in both freezing intolerant and freezing tolerant species, and LLT in the latter, also has a clear geographic component (Fig. 2.2a, b). In the northern hemisphere, for which most data are available, the minimum SCP recorded declines with latitude and a similar, though less well defined pattern is clear for LLT.

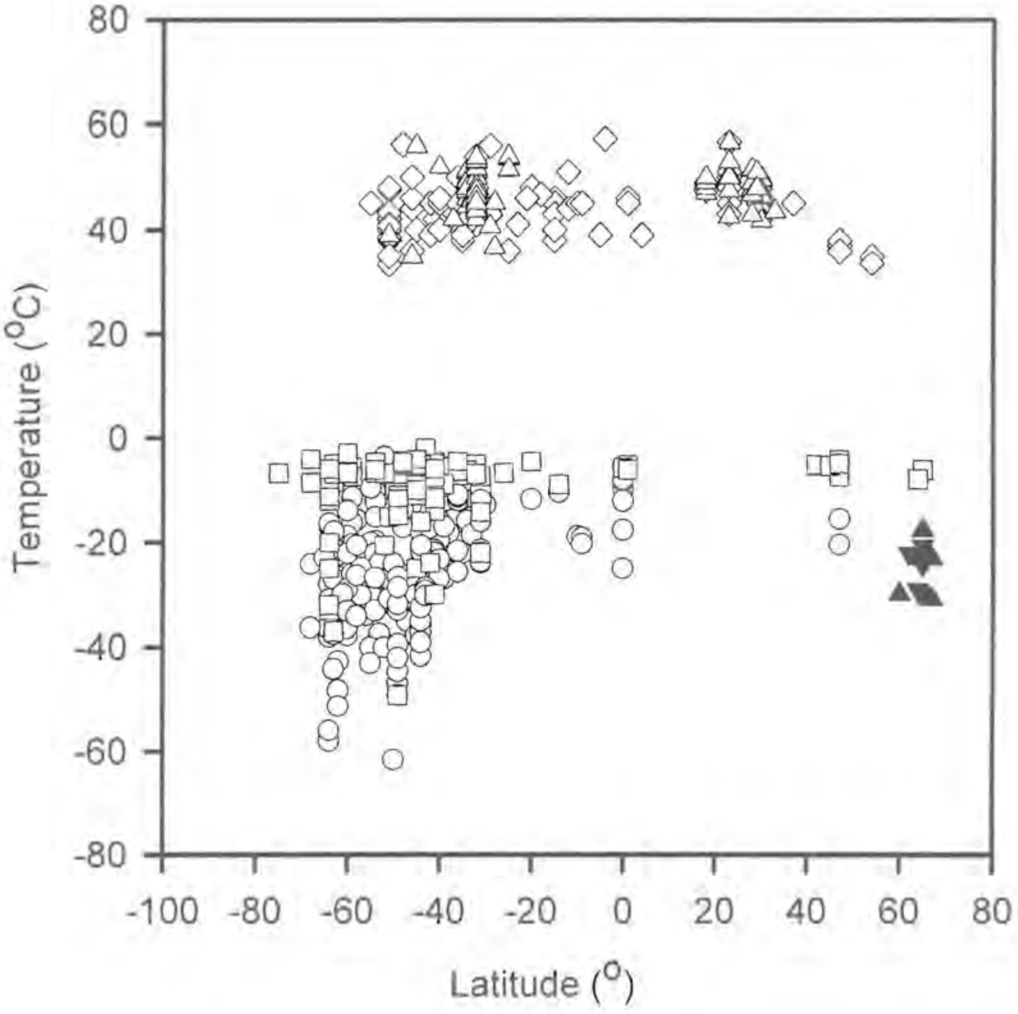

Figure 2.2. (a) Latitudinal variation (northern latitudes are negative) in supercooling points (SCP) (freezing intolerant (○) and freezing tolerant insect species (□)) and upper thermal limits (critical thermal maxima (Δ) and upper lethal temperatures (◊)). Data for some freezing intolerant Antarctic Collembola (▼) and Acari (▲).

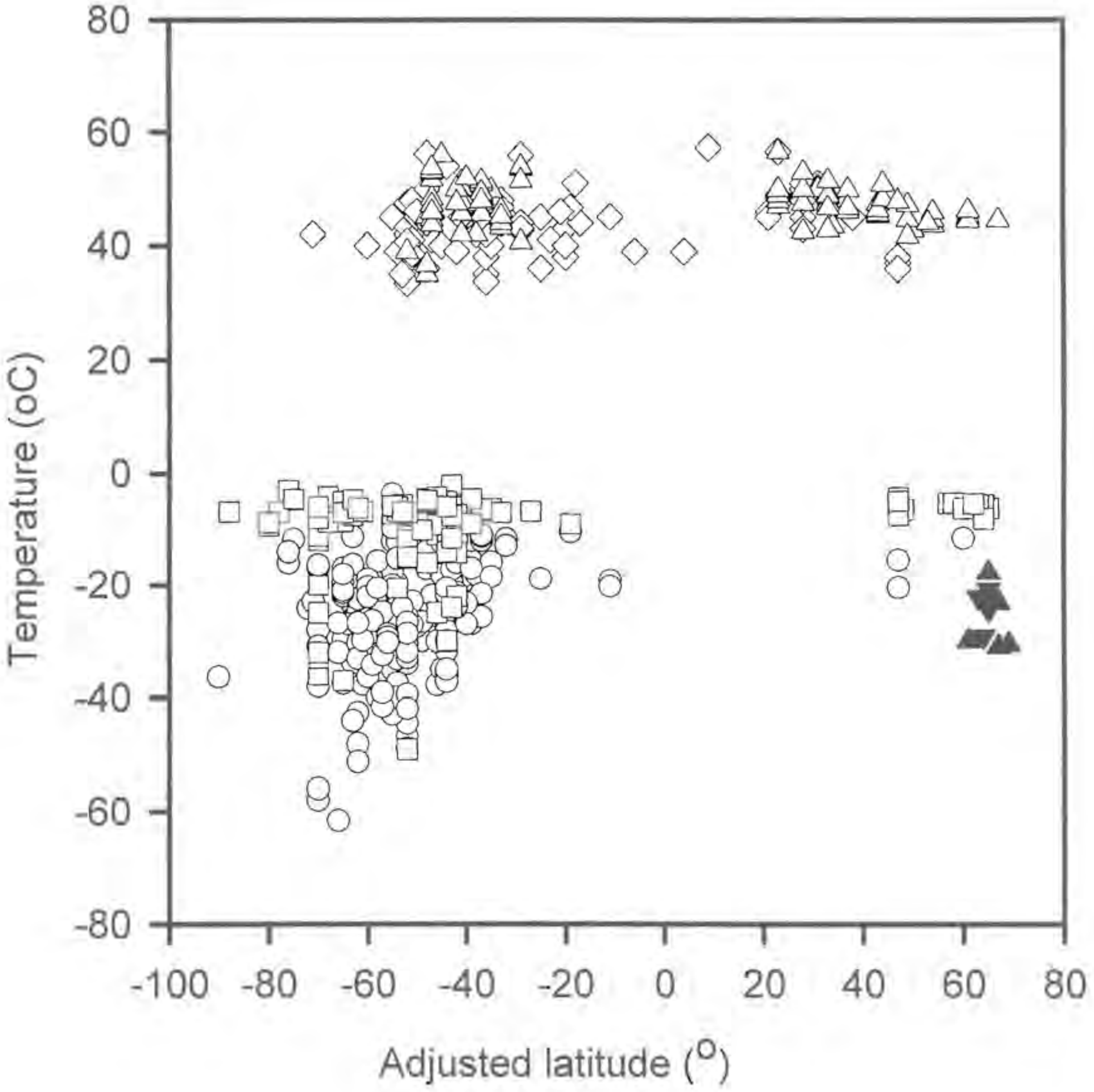

Figure 2.2. (c) Same data as in 2a, but the latitudinal position has been adjusted for altitude using Price *et al.*'s (1998) method.

The low values recorded in the temperate to tropical regions are generally representative of studies undertaken at high altitude. Indeed, if the localities at which these studies were undertaken are corrected to sea level, using a four degree increase in latitude for every 305 m increase in elevation (Price *et al.*, 1998), then it is clear that few studies have been done in the warm tropics (Fig. 2.2c). Whether these effects of altitude are included or ignored makes little difference to the outcome. That is, SCP and LLT tend to increase towards the equator. This latitudinal decline in SCP remains even when the data are resampled to nine data values per ten-degree latitudinal bin, above 30° N, to take account of the limited SCP data for species from the tropics (Fig. 2.3).

Although there were consistent differences between ULT (44.4 ± 0.34 °C, mean ± S.E.) and CTMax (47.4 ± 0.36 °C) ($F_{(1, 232)} = 26.57$, $p < 0.0001$), variation in upper thermal tolerance shows little geographic variation. Indeed, although there appears to be some decline in ULT in higher latitudes in the southern hemisphere, the general trend is towards similar high temperature tolerances across the globe (Fig. 2.2a), even when the data are adjusted for altitude (Fig. 2.2c).

These findings provide support for previous conclusions regarding latitudinal variation in insect cold hardiness that were based on limited interspecific (Kimura *et al.* 1994; Kukal *et al.*, 1991; Goto & Kimura, 1998) and intraspecific comparisons (Kukal & Duman, 1989; Heinze *et al.*, 1998). They are likewise supportive of the idea that upper thermal limits show considerably less geographic variation than do lower ones (Vannier, 1994; Lutterschmidt & Hutchinson, 1997; Goto & Kimura, 1998; Gaston & Chown, 1999b). Thus there is substantial evidence in favour of the physiological tolerance assumption of the climatic variability hypothesis (Stevens, 1989; Gaston *et al.*, 1998).

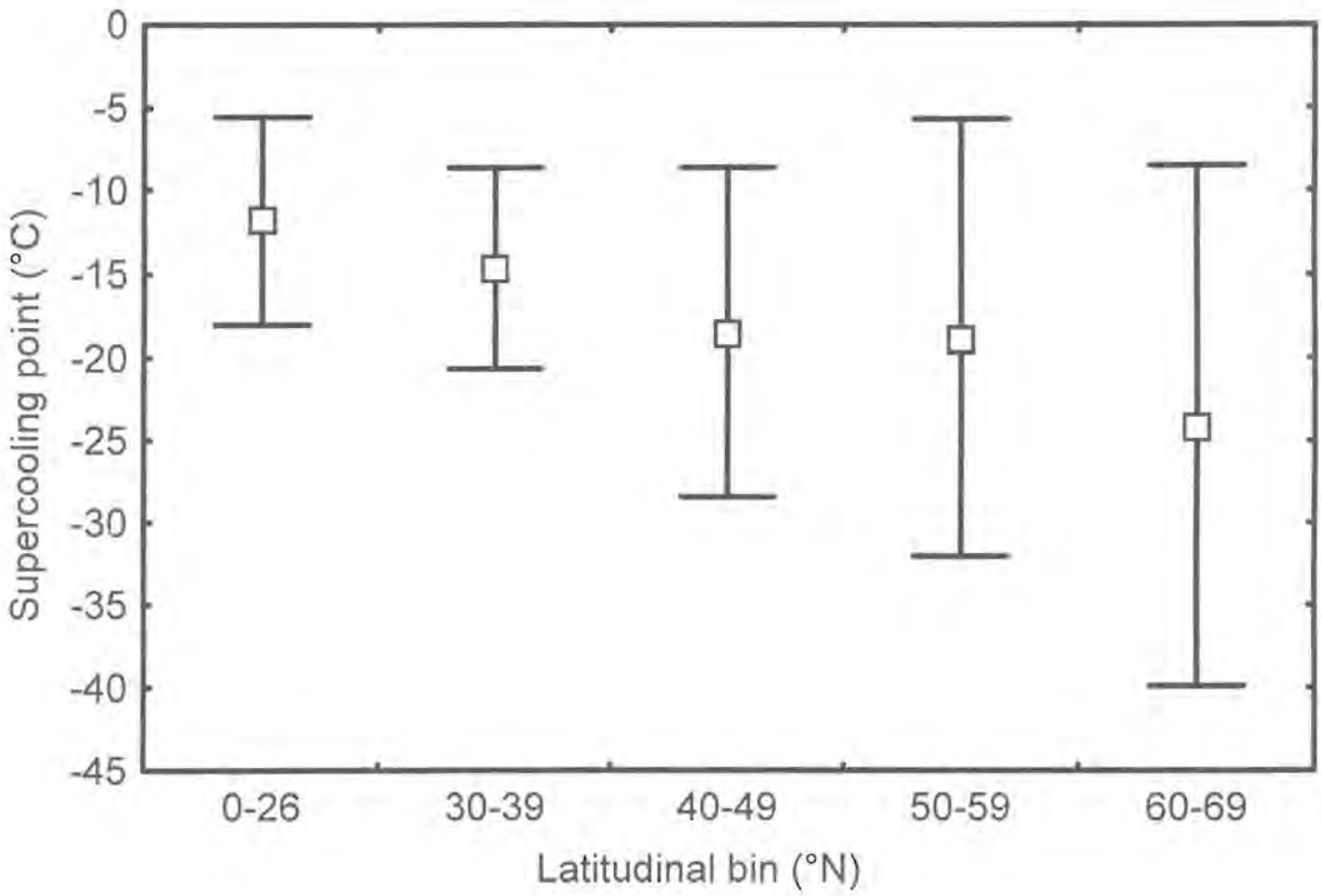

Figure 2.3. Mean ± S.D. of supercooling point (SCP) for each of five, 10° latitudinal bins in the northern hemisphere, after the original SCP data were resampled to nine data values per 10° bin, ten times (SCP and latitudinal bin mean $r_s = -0.91$, $p < 0.05$, $n = 9$ per bin). The relationship is in the same direction, but not significant if resampling is based on freezing intolerant species only (mean $r_s = -0.8$, $p = 0.12$, $n = 5$ per bin).

Indeed, a comparison of the absolute maximum and minimum temperatures reported for the New World (Fig. 2.4) with the lower bound of the SCP and LLT plots (Fig. 2.2), and the upper bound of the upper lethal temperature plots indicates a close correspondence between the two. However, the decline in SCP and, to some extent, LLT is not shown by all species. Rather, SCP and LLT variation tends to increase with latitude using both the original data (Fig. 2.2; for freezing intolerant species, standard deviation of SCP and latitude using 10° latitudinal bins, $r_s = 0.9$, $p < 0.05$), and the SCP values resampled, ten times, to nine (all data) data points per 10° latitudinal bin in the northern hemisphere (all data: Fig. 2.3 - standard deviation of SCP and latitude using 10° latitudinal bins mean $r_s = 0.9$, $p < 0.05$, $n = 9$ per bin). For five data points (freezing intolerant species only) the relationship is in the same direction, but it is not significant (mean $r_s = 0.7$, $p = 0.2$, $n = 5$ per bin), largely as a consequence of small sample sizes.

A variety of mechanisms could potentially account for this pattern. First, variation in the protocols adopted by researchers will undoubtedly have affected the outcome of their experiments. Generally, more rapid experimental cooling rates tend to lower insect survival and elevate SCPs (e.g. Kelty & Lee, 1999). However, the extent of this variation is considerably less than that documented here. A second, partially artefactual, mechanism could be latitudinal variation in researcher effort. The number of studies done and hence the number of taxonomic groups assessed tends to increase with latitude. Hence, variation in SCP and LLT should increase because much of it is partitioned at higher taxonomic levels.

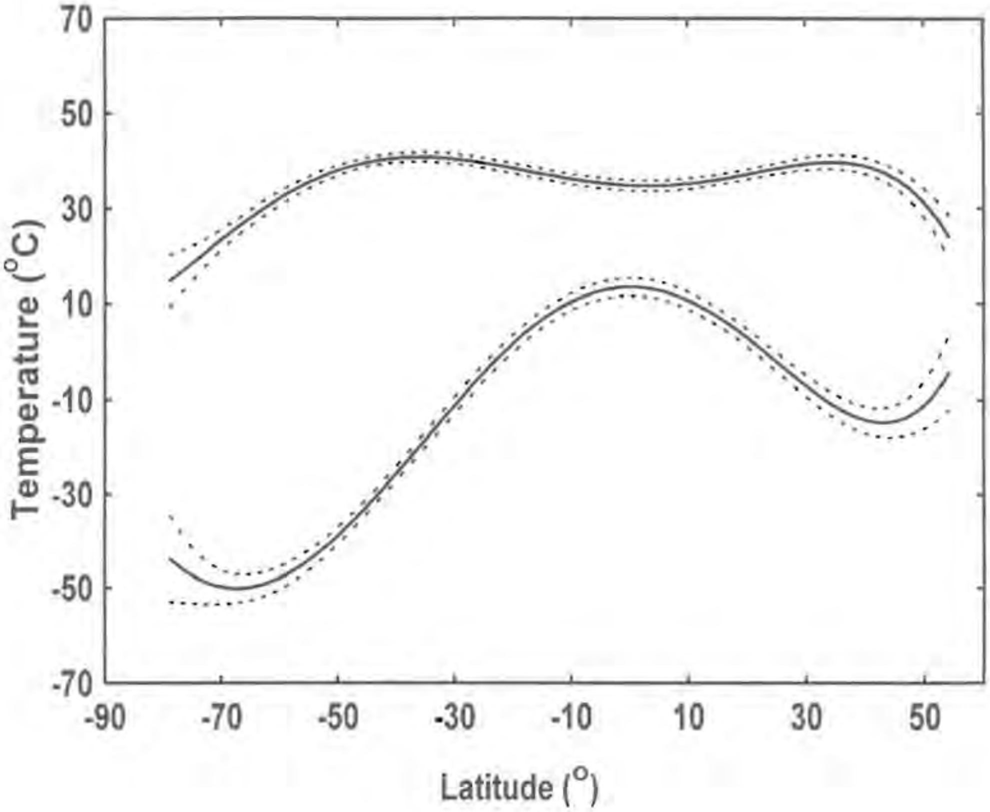

Figure 2.4. Best fit polynomial regression lines (±95%) showing the relationship between latitude and absolute maximum (top line) and absolute minimum (bottom line) temperatures across the New World (negative latitudes are north of the equator) (data from Müller, 1982, redrawn from Gaston & Chown, 1999b).

Although this is the case for the SCP of freezing intolerant species (for 10 degree latitude bins there is a positive relationship between mean latitude and mean number of families examined, $r_s = 0.793$, $p = 0.033$), such a relationship is not present in the freezing tolerant species ($p > 0.11$). In addition, this mechanism cannot account for the fact that some insects with apparently high SCPs and LLTs can survive the apparently low temperatures recorded at high latitudes. Rather, the increase in variation with latitude suggests that the variety of temperatures and situations either promoting or reducing the risks of low temperature injury encountered by insects (see Bale, 1987) must increase with latitude. For example, at those latitudes where there is a significant accumulation of snow, species overwintering in subnival habitats are likely to encounter less extreme temperatures than those in more exposed habitats (Danks, 1996). Finally, some of this variation may be due to the length of time an insect is likely to encounter subzero temperatures and conditions leading to inoculative freezing, factors that have a pronounced effect on insect cold hardiness (Sømme, 1996, 1999; Storey & Storey, 1996), but which were not explicitly controlled for in this study. Pronounced low temperature tolerance may develop in response to chronic exposure to moderately low temperatures, rather than as a response to an acute bout of extremely low temperature.

Nonetheless, the general pattern of upper and lower temperature tolerances is supportive of the physiological tolerance assumption, and particularly so in the northern hemisphere above approximately 20-30°N. The latter finding provides additional, strong evidence for the physiological tolerance assumption because it is in this region that the Rapoport effect is most evident and consistent across taxa (Rohde, 1996; Gaston *et al.*, 1998; Gaston & Chown, 1999a).

An additional pattern that emerged from the analysis of geographic variation in SCP and LLT was north-south asymmetry in this variation, even when data for freezing intolerant Antarctic mites and collembolans were included because of the almost complete absence of insects from this continent (Fig. 2.2). This variation also clearly paralleled the north-south variation in absolute minimum temperatures (Fig. 2.4), which in turn is undoubtedly a consequence of the vast extent of the southern oceans, and large area covered by the northern land masses (Klok & Chown, 1997; Gaston & Chown, 1999a, b). It has also been suggested that the generally oceanic nature of the southern hemisphere may promote the evolution of freezing tolerance because of the enhanced potential for inoculative freezing in moist habitats (Klok & Chown, 1997). The current analysis certainly bears out this suggestion, but the paucity of the data for the southern hemisphere leaves some doubt as to its generality. Nonetheless, north-south asymmetry in lower lethal temperatures and cold hardiness strategy is an important macrophysiological pattern, that seems to vary in a way similar to temperature. The latter may well account for the general lack of a Rapoport effect in vertebrates and plants in the southern hemisphere (Gaston & Chown, 1999a), and its effect on insect cold hardiness might well be expected to result in a similar effect.

Whilst ignoring a variety of factors known to affect lower and upper lethal temperature limits, this assessment of geographic variation in insect thermal tolerances reveals several important patterns. First, despite many statements that tropical insects cannot tolerate low temperatures, the data are largely lacking. Second, freezing tolerance appears to predominate in the southern hemisphere, but here too data are scarce. Third, upper lethal limits show much less variation than do lower lethal limits. Finally, and most significantly, there is considerable, though qualified support for the physiological tolerance assumption of the climatic variability hypothesis proposed to account for Rapoport's rule, especially above 20-30°N. Indeed, the substantial variation found in insect lower lethal temperatures, within latitudinal bands, suggests that while physiological tolerances in some species vary in the direction predicted by this assumption, others do not. Stevens (1989) implied that the physiological tolerance assumption might not apply to insects that effectively escape the extremes of climate in the overwintering stage. To some extent this does appear to be the case. However, the more crucial question is whether this variation in physiological tolerance translates to variation in range size. To date few studies have systematically examined geographic variation in insect latitudinal extents, and the few that have do not usually include any information on physiological tolerances.

References

Bale, J. S. 1987. Insect cold hardiness: Freezing and supercooling - an ecophysiological perspective. *Journal of Insect Physiology* **33**, 899-908.

Bale, J. S. 1993. Classes of insect cold hardiness. *Functional Ecology* **7**, 751-753.

Bale, J. S. 1996. Insect cold hardiness: A matter of life and death. *European Journal of Entomology* **93**, 369-382.

Blackburn, T. M. & Gaston, K. J. 1996. Spatial patterns in the geographic range sizes of bird species in the New World. *Philosophical Transactions of the Royal Society of London B* **351**, 897-912.

Block, W. 1982. Cold hardiness in invertebrate poikilotherms. *Comparetive Biochemistry and Physiology* **73A**, 581-592.

Block, W. 1990. Cold tolerance of insects and other arthropods. *Philosophical Transactions of the Royal Society of London B* **326**, 613-633.

Chown, S. L., Le Lagadec, M. D. & Scholtz, C. H. 1999. Partitioning variance in a physiological trait: desiccation resistance in keratin beetles (Coleoptera, Trogidae). *Functional Ecology* **13**, 838-844.

Danks, H. V. 1996. The wider integration of studies on cold hardiness. *European Journal of Entomology* . **93**, 383-403.

Duman, J. G., Wu, D. W., Xu, L., Tursman, D. & Olsen, T. M. 1991. Adaptations of insects to subzero temperatures. *Quarterly Review of Biology* **66**, 387-410.

Feder, M. E. & Hofmann, G. E. 1999. Heat-shock proteins, molecular chaperones, and the stress response – evolutionary and ecological physiology. *Annual Review of Physiology* **61**, 243-282.

Fleishman, E., Austin, G. T. & Weiss, A. D. 1998. An empirical test of Rapoport's rule: elevational gradients in montane butterfly communities. *Ecology* **79**, 2482-2493.

Gaston, K. J., Blackburn, T. M. & Spicer, J. I. 1998. Rapoport's rule: time for an epitaph? *Trends in Ecology & Evolution* **13**, 70-74.

Gaston, K. J. & Chown, S. L. 1999a. Why Rapoport's rule does not generalise. *Oikos* **84**, 309-312.

Gaston, K. J. & Chown, S. L. 1999b. Elevation and climatic tolerance: a test using dung beetles. *Oikos* **86**, 584-590.

Goto, S. G. & Kimura, M. T. 1998. Heat- and cold-shock responses and temperature adaptations in subtropical and temperate species of *Drosophila*. *Journal of Insect Physiology* **44**, 1233-1239.

Harvey, P. H. & Pagel, M. D. 1991. *The Comparative Method in Evolutionary Biology*. Oxford, Oxford University Press.

Heinze, J., Foitzik, S., Kipyatkov, V. E. & Lopatina, E. B. 1998. Latitudinal variation in cold hardiness and body size in the arboreal ant species, *Leptothorax acervorum* (Hymenoptera: Formicidae). *Entomologia Generalis* **22**, 305-312.

Johnson, C. N. 1998. Rarity in the tropics: latitudinal gradients in distribution and abundance in Australian mammals. *Journal of Animal Ecology* **67**, 689-698.

Kelty, J. D. & Lee, R. E. 1999. Induction of rapid cold hardening by cooling at ecologically relevant rates in *Drosophila melanogaster*. *Journal of Insect Physiology* **45**, 719-726.

Kimura, M. T., Ohtsu, T, Yoshida, T., Awasaki, T. & Lin, F-J. 1994. Climatic adaptations and distributions in the *Drosophila takahashii* species subgroup (Diptera: Drosophilidae). *Journal of Natural History* **28**, 401-409.

Klok, C. J. & Chown, S. L. 1997. Critical thermal limits, temperature tolerance and water balance of a Sub-Antarctic caterpillar, *Pringleophaga marioni* (Lepidoptera: Tineidae). *Journal of Insect Physiology* **43**, 685-694.

Klok, C. J. & Chown, S. L. 1998. Interactions between desiccation resistance, host-plant contact and the thermal biology of a leaf-dwelling sub-antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). *Journal of Insect Physiology* **44**, 615-628.

Knight, J. D., Bale, J. S., Franks, F., Mathias, S. F. & Baust, J. G. 1986. Insect cold hardiness: supercooling points and pre-freeze mortality. *Cryoletters* **7**, 194-203.

Kukal, O. & Duman, J. G. 1989. Switch in overwintering strategy of two insect species and latitudinal differences in cold hardiness. *Canadian Journal of Zoology* **67**, 825-827.

Kukal, O., Ayres, M. P. & Scriber, J. M. 1991. Cold tolerance in relation to the distribution of swallowtail butterflies. *Canadian Journal of Zoology* **69**, 3028-3037.

Lawton, J. H. 1999. Are there general laws in ecology? *Oikos* **84**, 177-192.

Lee, R. E & Delinger, D. L. 1991. *Insects at Low Temperature*. New York, Chapman and Hall.

Lee, R. E., Lee, M. R. & Strong-Gunderson, J. M. 1993. Insect cold hardiness and ice nucleating active microorganisms including their potential use for biological control. *Journal of Insect Physiology* **39**, 1-12.

Lutterschmidt, W. I. & Hutchison, V. H. 1997. The critical thermal maximum: history and critique. *Canadian Journal of Zoology* **75**, 1561-1574.

Müller, M. J. 1982. *Selected Climatic Data for a Global Set of Standard Stations for Vegetation Science*. The Hague, Junk.

Price, P. W., Fernandes, G. W., Lara, A. C. F. & Brawn, J. 1998. Global patterns in local number of insect galling species. *Journal of Biogeography* **25**, 581-591.

Read, A. F. & Harvey, P. H. 1989. Life history differences among the eutherian radiations. *Journal of Zoology London* **219**, 329-353.

Ring, R. A. 1982. Freezing tolerant insects with low supercooling points. *Comparative Biochemistry and Physiology* **73A**, 605-612.

Rohde, K. 1992. Latitudinal gradients in species diversity: the search for the primary cause. *Oikos* **65**, 514-527.

Rohde, K. 1996. Rapoport's rule is a local phenomenon and cannot explain latitudinal gradients in species diversity. *Biodiversity Letters* **3**, 10-13.

Roy, K., Jablonski, D. & Valentine, J. W. 1994. Eastern Pacific molluscan provinces and latitudinal diversity gradient: no evidence for 'Rapoport's rule'. *Proceedings of the National Academy Science of the United States of America* **91**, 8871-8874.

Roy, K., Jablonski, D., Valentine, J. W. & Rosenberg, G. 1998 Marine latitudinal diversity gradients: tests of causal hypotheses. *Proceedings of the National Academy Science of the United States of America* **95**, 3699-3702.

Ruggiero, A. & Lawton, J. H. 1998. Are there latitudinal and altitudinal Rapoport effects in the geographic ranges of Andean passerine birds? *Biological Journal of Linnean Society* **63**, 283-304.

Santelices, B. & Marquet, P. A. 1998. Seaweeds, latitudinal diversity patterns, and Rapoport's Rule. *Diversity and Distributions* **4**, 71-75.

Scholander, P. F., Flagg, W., Walters, V. & Irving, L. 1953. Climatic adaptation in Arctic and tropical poikiliotherms. *Physiological Zoology* **26**, 67-92.

Sinclair, B. J. 1999. Insect cold tolerance: How many kinds of frozen? *European Journal of Entomology* **96**, 157-164.

Sinclair, B. J., Worland, M. R. & Wharton, D. A. 1999. Ice nucleation and freezing tolerance in New Zealand alpine and lowland weta, *Hemideina* spp. (Orthoptera: Stenopelmatidae). *Physiological Entomology* **24**, 56-63.

Sømme, L. 1982. Supercooling and winter survival in terrestrial arthropods. *Comparative Biochemistry and Physiology* **73A**, 519-543.

Sømme, L. 1996. The effect of prolonged exposures at low temperatures in insects. *Cryoletters* **17**, 341-346.

Somme, L. 1999. The physiology of cold hardiness in terrestrial arthropods. *European Journal of Entomology* **96**, 1-10.

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

Stevens, G. C. 1989. The latitudinal gradient in geographical range: how so many species coexist in the tropics. *American Naturalist* **133**, 240-256.

Stevens, G. C. 1992. The elevational gradient in altitudinal range: an extension of Rapoport's latitudinal rule to altitude. *American Naturalist* **140**, 893-911.

Storey, K. B. & Storey, J. M. 1996. Natural freezing survival in animals. *Annual Review of Ecology and Systematics* **27**, 365-386.

Vannier, G. 1994. The thermobiological limits of some freezing intolerant insects: The supercooling and thermostupor points. *Acta Oecologica* **15**, 31-42.

Zachariassen, K. E. 1985. Physiology of cold tolerance in insects. *Physiological Reviews* **65**, 799-832.

Chapter 3

Revisiting water loss in insects: a large scale view

Accepted for publication in *Journal of Insect Physiology* (2001)

3.1 Introduction

Temperature and water availability are arguably the two most important abiotic variables influencing the distribution and abundance of terrestrial organisms. This is true also of insects (Chown & Gaston, 1999), which, because of their small body size, are particularly vulnerable to water loss. In consequence, differences in the ways insect species achieve water balance in a variety of different habitats have been extensively investigated Edney, 1977; Hadley, 1994; Sømme, 1995). Much of this work has been done on one, two or just a few species (Ahearn & Hadley, 1969; Ahearn, 1970; Eckstrand & Richardson, 1981; Juliano, 1986; Naidu & Hattingh, 1988; Chown *et al*., 1995; Klok & Chown, 1998) or populations (Hadley & Massion, 1985), although multi-species studies are being undertaken more commonly (Davidson, 1990; Hood & Tschinkel, 1990; Chown, 1993; Gereben, 1995; Le Lagadec *et al*., 1998). Virtually all of these studies have been concerned with demonstrating greater desiccation resistance or tolerance in species from arid environments.

Synthetic works have generally used a selection of data from these studies to argue, though usually without explicit analysis of environmental variables, that species from arid environments do indeed show lower rates of water loss, while maximum tolerable water loss tends to be much less variable across species. An early analysis of this kind prompted Edney (1977) to draw the useful distinction between a water balance strategy aimed primarily at reducing water loss rates, termed desiccation resistance, and one where loss of water is tolerated, known as desiccation tolerance. Edney (1977) also distinguished between species that behaviourally avoid desiccating conditions, and those that resist or tolerate them. Taking this classification an important step further, Hadley (1994) proposed five adaptive types, or models:

behavioural avoidance; enhanced water conservation; dehydration tolerance; high fluid turnover; water sorption dependence. This scheme continues to serve an important role in promoting an understanding of the diversity of physiological strategies arthropods employ to cope with differences in water availability. However, like similar models (e.g. Bale, 1993), which have proved useful and important in guiding insect physiology toward new insights, Hadley's (1994) models remain largely explanatory. As such they address only one of science's major goals, leaving the other, predictability (Nagel, 1961; Casti, 1991), largely unattended.

However, shortly after Hadley's (1994) scheme was published, Zachariassen (1996) developed a more restricted, but quantitative model for water balance in insects, based on earlier work he had undertaken (Zachariassen *et al.*, 1987; Zachariassen *et al.*, 1988). Building on relationships between body mass, metabolic rate, and rate of water loss, Zachariassen (1996) argued that insects from arid environments would show very little cuticular transpiration and, in some cases, have lower metabolic rates than those of the same size from mesic habitats, and consequently that their rates of water loss would be lower. Furthermore, he suggested that in plant-feeding groups, such as tenebrionids, reduced metabolic rates would mean reduced extracellular sodium concentrations, but elevated extracellular free amino acid concentrations, while this would not be the case in predators, such as carabid beetles. Thus, according to the Zachariassen (1996) model (ZM), if the functional or taxonomic group of an insect, and its body mass are known, several predictions concerning its water balance characteristics under different environmental scenarios can be made.

Not only is the ZM intriguing, but it also appears to have independent support. For example, in their investigation of water loss in a sub-Antarctic caterpillar, Klok &

Chown (1998) found that their data on this highly desiccation resistant species supported the model, despite the fact that a larval lepidopteran, rather than a beetle, was being investigated. Indeed, Klok & Chown (1998) included several non-coleopterans in their comparison and concluded that the ZM was useful for a broad range of insect taxa.

Nonetheless, the ZM appears to be compromised by several recent findings regarding the contribution to water loss of respiratory transpiration. Using flow-through gas analysis, several authors have partitioned transpiration into that portion occurring during the open spiracular phase of the discontinuous gas exchange cycle (see Lighton, 1996, 1998), and that portion taking place in the closed phase. It is assumed that the former represents both spiracular and cuticular transpiration, whereas the latter represents cuticular water loss only (Hadley & Quinlan, 1993). In those species (including some from xeric areas) that have been examined using these methods the contribution to total water loss of respiratory transpiration is typically in the order of only 5-10% (see Bosch *et al.*, 2000 for a summary of the studies). In contrast, Zachariassen (1996) suggested that while most mesic insects have high cuticular transpiration rates, in species from arid environments a substantial fraction of water lost occurs via respiration, because cuticular transpiration is much reduced (see also Zachariassen & Maloiy, 1989; Zachariassen, 1991). Indeed, it appears that for the ZM to enjoy support as a functional model of water loss, respiratory transpiration must account for a significant portion of water lost from arid-environment insects during dehydration. Therefore, the data presented by Zachariassen and his colleagues (Zachariassen *et al.*, 1987; Zachariassen *et al.*, 1988; Zachariassen & Maloiy, 1989; Zachariassen, 1991) seem to be at odds with those gathered using flow-through techniques, posing something of a conundrum.

In this study therefore the question of environmental effects on desiccation resistance in insects was revisited and the utility of Zachariassen (1996) model. The aims are threefold. First, using a database collated from the existing literature the environmental correlates of desiccation resistance were explicitly examined. Most studies have concluded that desiccation resistance shows such environmentally-associated variation without formally seeking an association between desiccation rate and one or more environmental variables (for exceptions see Eckstrand and Richardson, 1981; Marsh, 1988; Gibbs *et al.*, 1991; North, 1991; Lighton *et al.*, 1994; Salin *et al.*, 1999). In doing so the geographic extent of the data on desiccation resistance was also determined in insects because this provides some indication of the range of environments over which this kind of work has been undertaken. Second, using data both from the original ZM papers (Zachariassen *et al.*, 1987; Zachariassen *et al.*, 1988) and other work, collated in this study, the Zachariassen model was re-examined and tested independently. Finally, the database was used to determine whether variation in desiccation rate is partitioned at higher taxonomic levels, as the ZM suggests.

3.2 Material and methods

The relevant anglophone literature on insect water balance was assessed as far back as 1928, but with major emphasis placed on the last 50 years. Over 300 published papers were examined. The following studies were excluded from the analyses: (i) Those dealing with insects pretreated either by applying a chemical or abrasive (e.g. aluminium dust or peanut oil); (ii) Experiments using decapitated or dead insects; (iii) Insects reared for several generations in the laboratory. These treatments are all known to affect the rate of water loss (Collins & Richards, 1966; Le Lagadec, 1997). The data collected were used for a determination of the geographic coverage of water balance studies, plotting study sites using Worldmap IV software (each grid cell approximately 611,000km^2; Williams, 1998). Data from studies where factors such as trial temperature and humidity were not provided were also excluded, and those that did not report water loss rates. Because data from adult insects predominated (>70%), the other developmental stages were excluded from the analysis. Following this selection, a second, iterative protocol was employed (see also Addo-Bediako *et al.*, 2000). Where a species was examined more than once, either in a single paper or by different authors, the record with a treatment temperature either of, or as close to 24°C was selected and the other records were discarded. However, if a species was entered twice or more with the same temperature, then the record from the geographic location that was least represented in the database was selected. Where the geographical location of the species was not stated in the original paper, and could not source an approximate range centre for the species from the literature, the location of the study was used (this was necessary in only a few instances). Thus, for the quantitative analysis of the relationship between water loss rate and environmental parameters, and for the nested analysis of variance (see below) water loss data on 180 species belonging to 21 families and 7 orders were extracted from a total of 76 published papers.

For each value of water loss the following corresponding data were included, temperature and humidity of the trial, geographic location (converted to latitude and longitude using either the grid reference provided in the original paper or the stated collection locality and gazetteers), initial body mass, and wing status (flightless or flying). In many cases the latter information had to be gathered either from the taxonomic literature, in consultation with taxonomic experts, or from the authors of the papers themselves. Despite a large literature suggesting that brachyptery/aptery plays an important role in reducing water loss, especially in beetles (Hesse, 1938; Cloudsley-Thompson, 1964; Ahearn, 1970; Scholtz, 1981; Draney, 1993; Chown *et al.*, 1998), the wing status of insects is often not reported in water balance studies.

Because water loss rate is reported in several different units, all data were converted to $g.h^{-1}$. In those studies where rates of water loss were given as a function of surface area, the formula, $S = kM^{0.667}$ was used, where S is the surface area, M is the initial mass and k represents a species- or group-specific constant (Edney, 1977) to back transform the data to $g.h^{-1}$. In the conversion, the k value provided in the original paper was used, though in a few studies k was not reported, and the mean value of k reported in other papers for species of the same family was used. Similarly, if authors corrected for saturation deficit (see Hadley, 1994), the rate of loss was back transformed so as not to include this correction.

A generalized linear model (McCullagah & Nelder, 1989) was used to investigate the strength and direction of associations between $\log_{10}$ rate of water loss, and $\log_{10}$ initial body mass, saturation deficit of the trial, wing status, environmental temperature, and environmental precipitation. For each data point environmental temperature and precipitation were determined by assigning a value, based on geographic position, from the half-degree mean annual temperature, and half-degree mean daily precipitation database (1961-1990) provided by the Intergovernmental Panel on Climate Change Data Distribution Centre (http://ipcc-ddc.cru.uea.ac.uk/ipcc_ddc/index.html). The exceptions were values for sub-Antarctic Marion and South Georgia Islands, where information was obtained from Schulze (1971) and Lewis Smith and Walton (1975) respectively, and for Spitsbergen, where values were obtained from www.svalbard.com/weather.html.

Nested analysis of variance using the Satterthwaite approximation for unequal sample sizes (Sokal & Rohlf, 1995: 300) was used to partition variance in water loss between the species (including the error term), generic, familial, and ordinal levels (see Le Lagadec *et al.*, 1998; Addo-Bediako *et al.*, 2000 for rationale).

To test Zachariassen (1996) model two main approaches were adopted. First, by reinvestigating the relationship between $\log_{10}$ water loss rate ($g.h^{-1}$) and $\log_{10}$ metabolic rate ($mm^3\ O_2.min^{-1}$) (hereafter WLRMR), using the data for xeric beetle species reported in Zachariassen *et al.* (1987) and Zachariassen *et al.*, (1988), and least squares linear regression analyses. The metabolic rates were then converted to μW in order to use values for metabolic rate for other species reported in the literature (and compiled by in a separate study – Addo-Bediako *et al.* submitted ms.). To ensure that the results using both units were consistent analysis of covariance was used to compare the slopes of the $\log_{10}$ metabolic rate - $\log_{10}$ desiccation rate relationships obtained using the two different units. Following this, the WLRMR relationship was investigated, using least squares linear regression, for those insect species (irrespective of order) not used for constructing the ZM model and for which both water loss and metabolic rate data were available. Using analysis of covariance the slope of the ZM WLRMR relationship was compared with that calculated independently here. Finally, the WLRMR relationship was investigated for mesic species. In this case, however, the data reported by Zachariassen (Zachariassen *et al.*, 1987; Zachariassen *et al.*, 1988) were pooled and the data that were collected independently because regression models were non-significant when the two sets of data were analyzed separately.

3.3 Results

Although the coarse resolution of the map (Fig. 3.1) does not allow a careful division of studies into those undertaken in mesic and xeric areas, it does provide some suggestion that much water balance work has been undertaken in desert regions (see Sømme, 1995, especially Fig. 1.1 for a designation of desert areas). Nonetheless, the geographic, and hence climatic coverage of this data compilation is considered to be sufficient for meaningful quantitative analyses of the association between environmental effects and water loss rates.

In the generalized linear model, both body mass and trial saturation deficit showed the expected significant, positive relationships with water loss rate (Table 3.1). The estimate for the slope of the relationship between mass and water loss rate was 0.818 ± 0.0765, which is significantly different (Student's t, $p < 0.001$, see Sokal and Rohlf, 1995: 471) from water loss expected on the basis of geometric considerations alone (i.e. a slope of 0.667). Because the use of saturation deficit in water loss studies is contentious (see Discussion) the analysis was repeated including both trial temperature and humidity, rather than saturation deficit. Here it was found that temperature had a larger effect than humidity on water loss rates, though both remained significant (Table 3.1). Most importantly, the inclusion of temperature and humidity, rather than just saturation deficit, had little effect on the significance of the other parameters in the model. Thus, the interpretation of the model was continued including the correction for saturation deficit only. In this model, wing status also influenced water loss rates (Table 3.1), with flightless species having lower rates (corrected mean = 0.406 $mg.h^{-1}$) than those with wings (corrected mean = 2.174 $mg.h^{-1}$), and of the environmental parameters, only precipitation entered the model

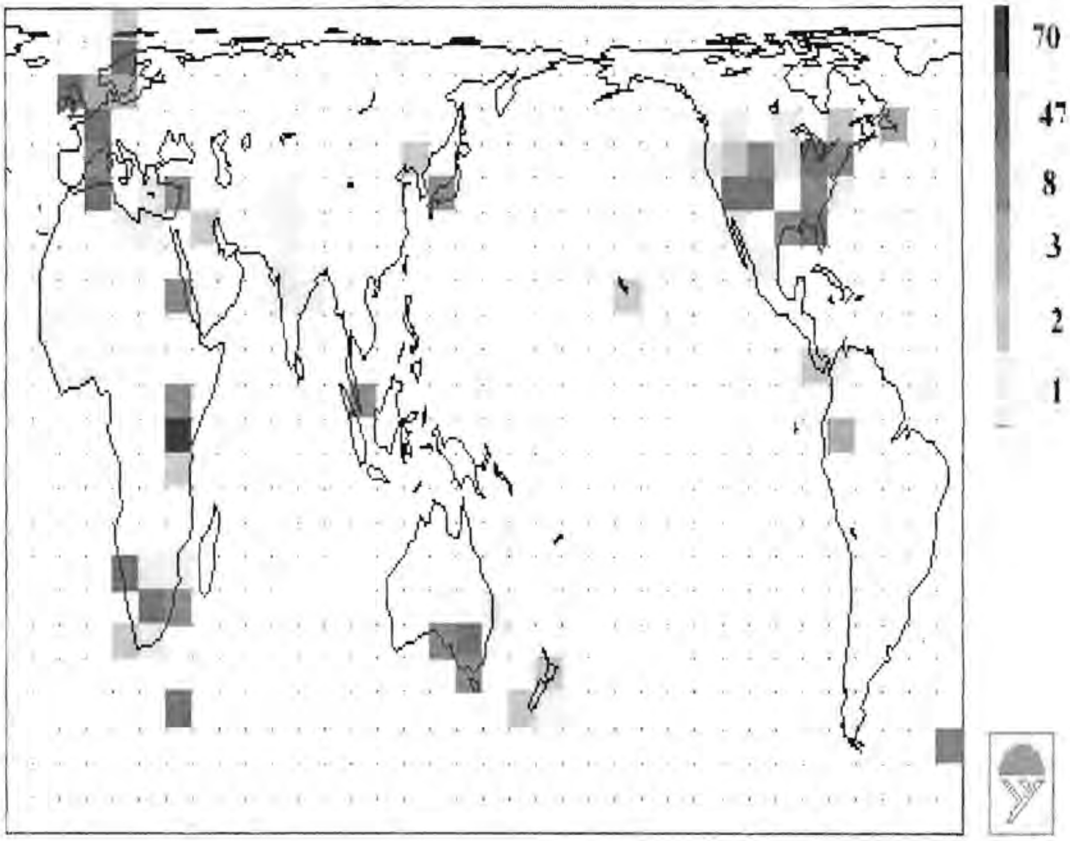

Fig. 3.1. Global distribution of studies reporting water loss rates for insects produced using WorldMap IV (Williams, 1998). The projection is Peters Equal Area and each grid cell is approximately 611 000 km^2

significantly (Tables 3.1 and 3.2). Where precipitation is highest, water loss rates also tend to be highest, thus quantitatively confirming the idea that water loss rates are significantly influenced by habitat aridity.

Table 3.1 Generalized linear model of the explanatory variables on $\log_{10}$ water loss rate including both trial temperature and humidity.

Variables Goodness of fit	Parameter estimate	df (df)	Type III log-likelihood (Deviance)	Chi-square (Deviance/df)	p
Log$_{10}$ mass	0.854	1	-260.2	101.8	0
Trial temperature	0.057	1	-224.4	30.2	0.000001
Trial humidity	0.021	1	-212.3	5.97	0.015
Ambient temperature		1	-210.4	2.20	0.138
Ambient precipitation	0.023	1	-220.4	22.3	0.000002
Wing status	0.854	1	-212.0	5.30	0.021
		(173)	(107.9)	(0.623)	

Table 3.2 Generalized linear model of the explanatory variables on $\log_{10}$ water loss rate for the coleopteran and non-coleopteran species analysed separately.

Variables Goodness of fit	Parameter estimate	df (df)	Type III log-likelihood (Deviance)	Chi-square (Deviance/df)	p
Coleoptera					
Log$_{10}$ mass	1.029	1	-206.7	58.9	0.00001
Ambient temperature		1	-178.2	1.77	0.183
Ambient precipitation	0.023	1	-186.2	17.9	0.00003
Saturation deficit	0.343	1	-185.2	15.9	0.00007
Wing status		1	-182.7	10.8	0.001
		(135)	(102.7)	(0.756)	
Non-Coleoptera					
Log$_{10}$ mass	0.729	1	-42.1	60.8	0.00001
Ambient temperature		1	-11.9	0.393	0.531
Ambient precipitation	0.022	1	-14.6	5.9	0.016
Saturation deficit		1	-12.3	1.17	0.278
Wing status		1	-13.2	3.03	0.082
		(33)	(4.163)	(0.126)	

Variation in water loss rate was not only associated with both trial conditions, wing status and precipitation. Rather, it also showed significant taxonomic variation, with much of the variation in water loss rates being partitioned at the genus level and above (Table 3.3).

Table 3.3. Distribution of variance in water loss rate. Tabulated values are percentage of the total variance accounted for at each successive level. The species level includes the error term in the data. (** $p < 0.001$).

Trait/level	Species	Genus	Family	Order
Variance (%)	31.98	29.56**	24.51**	13.95

On re-examining the Zachariassen model a small, though non significant (Student's t, $p > 0.09$) difference between the slope of the WLRMR relationship that Zachariassen (1996) reported for the xeric species (0.902) was found, and the one calculated from his data for these species (Zachariassen *et al.*, 1987; Zachariassen *et al.*, 1988) (Table 3.4). Presumably, this difference is largely a result of rounding error in the raw data. On conversion of the original metabolic rate data to microwatts, no difference between the slope of the WLRMR regressions in the two data sets was found (ANCOVA $F_{(1, 26)} = 0.059$, $p = 0.810$, Table 3.4). Therefore, all further analyses were conducted using metabolic rate estimates converted to microwatts. Neither the slope (ANCOVA $F_{(1, 23)} = 0.079$, $p = 0.781$, Table 3.4) nor the intercept (ANCOVA $F_{(1, 24)} = 1.406$, $p = 0.247$, Table 3.4) of the relationship between water loss rate and metabolic rate, found using an independent data set for xeric species, differed from those obtained using the data provided by Zachariassen *et al.*, 1987; Zachariassen *et al.*, 1988). Although the slopes of the relationships between water loss rate and metabolic rate appeared to differ between the xeric and mesic species (Table 3.4), this was not the case (ANCOVA $F_{(1, 47)} = 1.031$, $p = 0.315$). Nonetheless, the intercepts were significantly different, with species from xeric environments tending to lose water at a significantly lower rate, for a given metabolic rate, than those from mesic environments (adjusted means: xeric species = 0.338 mg.h^{-1}, mesic species = 0.722 mg.h^{-1}; ANCOVA $F_{(1, 48)} = 6.674$, $p = 0.013$).

Table 3.4. Summary statistics for the least squares linear regressions examining the relationships between metabolic rate, water loss rate and body mass for mesic and xeric insects for which data are available on all three variables.

Variables	Slope S.E.	Intercept S.E.	F	df	p	R^2
Log$_{10}$ metabolic rate and log$_{10}$ water loss rate in ZM xeric species						
mm3 O_2.hr^{-1}	0.897 ± 0.045	-3.522 ± 0.029	382	13	0.0001	0.965
μW	0.868 ± 0.114	-5.663 ± 0.268	57	13	0.0001	0.802
Log$_{10}$ metabolic rate and log$_{10}$ water loss rate in other xeric species						
	0.940	-5.654 ± 0.663	16	11	0.002	0.560
Log$_{10}$ metabolic rate and log$_{10}$ water loss rate in the combined xeric species data set						
	0.941 ± 0.117	-5.759 ± 0.300	64	25	0.0001	0.709
Log$_{10}$ mass and log$_{10}$ water loss rate						
	0.678 ± 0.126	-3.018 ± 0.120	28	25	0.0001	0.517
Log$_{10}$ mass and log$_{10}$ metabolic rate						
	0.557 ± 0.125	2.815 ± 0.119	20	25	0.0002	0.420
Residuals of log$_{10}$ metabolic rate and log$_{10}$ water loss rate in the combined xeric species data set						
	0.720 ± 0.142	0 ± 0.066	26	25	0.0001	0.486
Log$_{10}$ metabolic rate and log$_{10}$ water loss rate in the combined mesic species data set						
	0.756 ± 0.133	-4.979 ± 0.334	32	22	0.0001	0.574
Log$_{10}$ mass and log$_{10}$ water loss rate						
	0.731 ± 0.109	-2.452 ± 0.146	45	22	0.0001	0.657
Log$_{10}$ mass and log$_{10}$ metabolic rate						
	0.767 ± 0.104	3.141 ± 0.139	55	22	0.0001	0.701
Residuals of log$_{10}$ metabolic rate and log$_{10}$ water loss rate in the combined mesic species data set						
			1.46	22	0.234	0.022

3.4 Discussion

Large-scale variation and its correlates

Positive relationships between water loss rate and both body mass and trial saturation deficit are not unexpected (see e.g. Edney, 1977; Chown, 1993; Hadley, 1994; Lighton *et al.*, 1994). Nonetheless, the difference between the slope of the mass and water loss rate found and that expected from geometric considerations alone is probably a consequence of the contribution of respiratory water loss to overall water loss (Zachariassen, 1991), and the possible incorporation of measurements of substrate catabolism in gravimetric analyses (see Nicolson, 1980; Edney, 1982 for discussion). However, the slope of the interspecific relationship between mass and rate of water loss does not always take the form found here. Rather, it varies substantially depending on the taxon studied. For example, Zachariassen *et al.* (1988) found a range of values (from 0.626 to 0.910) for East African beetles from different higher taxa and environments (mesic vs. xeric), and Chown (1993) found a slope as low as 0.484 for weevils from Marion Island. Somewhat unusually, a consensus scaling relationship for water flux in insects has not been presented in recent major reviews of water balance (Arlian & Veselica, 1979; Wharton, 1985; Hadley, 1994) or of scaling (Peters, 1983; Schmidt-Nielsen, 1984). This might be attributable to the difficulty of determining cuticular transpiration (see Edney, 1977; Nicolson *et al.*, 1984; Hadley, 1994), problems in determining overall transpiration corrected for catabolism, and/or the difficulty of accounting for the influence of temperature and saturation deficit on these measures (Edney, 1977, 1982). The development of sophisticated flow-through methods for measuring water loss (Lighton, 1991; Quinlan & Hadley, 1993; Bradley *et al.*, 1999) suggests that the development of a meaningful scaling relationship for water loss in insects might now be more readily achieved (though see below). Although this is by no means a trivial task, the utility of scaling relationships in both ecology and physiology (Peters, 1983, Schmidt- Nielson, 1984) would repay the effort.

Correction for saturation deficit, as done here, remains contentious in water balance studies, with some authors rejecting it (Toolson, 1978, 1980) and others continuing to recommend its use (Monteith & Campbell, 1980; Gelman *et al.*, 1988). Despite much discussion (see also Gilby, 1980; Gibbs, 1998), the jury still seems to be out on this issue. In the second model constructed, when saturation deficit was excluded, a larger effect of temperature than of humidity on water loss rates was found, though both were significant. If the table of transition temperatures for cuticular transpiration in insects provided by Gibbs (1998) is used as a yardstick, the majority of the data included in this study came from studies conducted below these temperatures. Consequently, it appears that differences in saturation deficit (which is influenced to a considerable degree by temperature), are a plausible explanation for at least part of the increase in water loss with temperature. The remainder of the increase must undoubtedly be due to the positive effects on metabolic rate of an increase in temperature. More significantly, the two models showed little difference in the behaviour of the other parameters, which were of considerably greater interest in this analysis.

While the significant effect of wing status immediately suggests that aptery/brachyptery has the predicted effect on water loss rates (Ahearn, 1970; Scholtz, 1981; Zachariassen, 1991; Draney, 1993; Hadley, 1994; Chown *et al.*, 1998), it should be noted that this argument has been applied solely to beetles. By virtue of the decline in the steepness of the diffusion gradient associated with the presence of a sub-elytral chamber, flightless beetles are thought to have a more restrictive water economy than winged ones (Hadley, 1994). Indeed, when this analysis was repeated, differentiating between beetles and non-beetle taxa, a significant and more pronounced effect of wing status on water loss rate in beetles was found (flying species corrected mean = 2.486 $mg.h^{-1}$, flightless species = 0.505 $mg.h^{-1}$), whereas the effect of wings disappeared in the non-coleopterans (Table 3.2). It should be noted, however, that the effect in the non-beetles verged on significance and the means differed in the predicted direction (flying species corrected mean = 1.075 $mg.h^{-1}$, flightless species = 0.230 $mg.h^{-1}$). Although the presence of a subelytral chamber might indeed mean lower water loss rates in flightless beetles, the reduction in water loss rate both in this group, and the indication thereof in the flightless non-coleopterans might also be a consequence of lower metabolic rates associated with non-flying species (Reinhold, 1999; Davis *et al*., 2000). If flightless species do indeed have reduced standard metabolic rates compared to those that routinely fly (Reinhold, 1999; Addo-Bediako *et al*. submitted ms.), and if water loss rates constitute some fixed proportion of the total water loss during rest (Bosch *et al*., 2000), then a reduction in metabolic rate in these species should contribute to water economy.

The finding that water loss rate is positively related to environmental precipitation, and is influenced by trial conditions and wing status, is likely to be compromised to some extent by the significant variation in water loss rates found above the species level in the nested analysis of variance. Such higher level variation means that species cannot be treated as independent units, thus influencing the strength of the statistical tests applied to the data (Felsenstein, 1985; Harvey & Pagel, 1991; Freckleton, 2000). However, in the absence of a phylogeny for the insects at the species level, and given the patchy distribution of desiccation tolerance work across families, it was decided not to include an independent contrasts analysis. In so doing, it is recognized that assumptions about the structure of the data are being made, but also note that several analyses have demonstrated consensus between the two methods (Ricklefs & Starck, 1996; Price, 1997). However, it must be admitted that an analysis using independent contrasts with a larger dataset would be useful.

Bearing the potential pitfall of phyloigenetic non-independence in mind, there is confirmation that water loss rates are related to precipitation (and hence habitat aridity) is important, especially because it demonstrates that over large scales physiological regulation remains sufficiently pronounced to be detected above the behavioural avoidance that is often important at smaller scales (Hadley, 1994; Sømme, 1995). In the absence of an analysis based on phylogenetic information (see e.g. Coddington, 1988; Baum & Larson, 1991) it is not clear whether desiccation resistance should be considered the derived condition in insects. The ZM suggests that this is the case, and this analysis thereof also supports this idea (see below). Nonetheless, based on work that has been undertaken to date it appears that desiccation resistance is a malleable trait that is likely to vary considerably between species within monophyletic taxa (Chown, 1993; Ward & Seely, 1996; Le Lagadec *et al.*, 1998).

The significant relationship between precipitation and water loss rate also lends weight to the argument that physiological tolerances are important in influencing species distribution pattern (see also Rogers & Randolph, 1991; Robinson *et al.*, 1997a, b; Chown & Gaston, 1999; Jenkins & Hoffmann, 1999; Parmesan *et al.*, 1999). At a time when the extent of the influence of climate on species distributions is being debated (Jeffree & Jeffree, 1994, Davis *et al.*, 1998; Holhberg & Ives, 1999; Chown & Clark, 2000), especially because of the importance of predicting species responses to climate change (Jeffree & Jeffree, 1996; Bryant *et al.*, 1997; Kerr & Packer, 1998), this finding is of considerable significance. While the current analysis suggests that changes in precipitation regime might have a significant influence on the birth and death rates of populations (and hence ultimately their geographic distributions), predicting future change in precipitation at the regional to local scales remains a significant obstacle for General Circulation Models (Schneider, 1992; Hewitson, 1997). Understanding the causal links between species physiological tolerances and their geographic distributions must therefore remain a priority on the global change agenda.

Testing the Zachariassen model

The absence of a significant difference in the relationship between water loss rate and metabolic rate found using an independent data and that of Zachariassen *et al.* (1987, 1988) appears to provide considerable independent support for the ZM. However, it might be also argued that the WLRMR relationship is simply the consequence of independent relationships between water loss rate and body mass, and metabolic rate and body mass (see above and also Lighton & Fielden, 1995; Davis *et al.*, 1999). To determine whether this is the case, the data and that provided by Zachariassen *et al.* (1987, 1988) were pooled. Least-squares linear regressions between body mass and metabolic rate and between body mass and water loss rate were then undertaken (Table 3.4). The relationship between the residuals from these regressions was examined using least squares regression, and was found to be significant, with a slope not significantly different from that obtained using the pooled, original data (Table 3.4, Student's t, $p > 0.1$). In consequence, the ZM appears to be well supported, at least so far as species from xeric environments are concerned.

However, the Zachariassen model is not predicated simply on the relationship between water loss rate and metabolic rate in xeric species. Rather, it also suggests that mesic species differ fundamentally from xeric ones. Although the slopes of the relationships between water loss rate and metabolic rate were not significantly different, the intercepts suggested that the mesic and xeric species differ fundamentally with xeric species showing the lower water loss rate at a given metabolic rate. More interestingly, the relationship between metabolic rate and water loss rate in the mesic species appears to be solely the consequence of an underlying relationship between these variables and body mass. In these species, there was no significant relationship between the residuals of the mass-metabolic rate and mass-water loss rate relationships (Table 3.4). Thus, the mesic and xeric species appear to differ profoundly, with water loss rates being substantially lower, for a given metabolic rate, in the xeric than in the mesic species. This idea is further borne out by the absence of a difference between the two groups of insects in their body mass - metabolic rate relationships (ANCOVA $F_{(1, 48)} = 1.43$, $p = 0.238$), but a pronounced difference in the body mass – water loss rate relationship (ANCOVA $F_{(1, 48)} = 14.6$, $p = 0.0004$), and the fact that after discounting the effects of body mass, there continues to be an underlying relationship between metabolic rate and water loss rate in the xeric, but not in mesic species.

In sum, metabolic rate and water loss rate appear to be a straightforward consequence of scaling in mesic species, whereas in xeric species this is not the case. In the latter, water loss rate is much reduced. Moreover, because metabolic rate is similar in xeric and mesic species of a similar size, respiratory transpiration in xeric species must constitute a greater proportion of total water loss than can be expected in a mesic species of a similar size. In consequence, a relationship between water loss rate and metabolic rate independent of that associated with body size can be expected. Such a difference between xeric and mesic species forms the basis for the Zachariassen (1996) model, which clearly enjoys substantial, independent support, and also raises several important issues that remain to be addressed.

Implications

Current evidence suggests that respiratory transpiration does not constitute a larger proportion of overall water loss in xeric than in mesic species (Hadley & Quinlan, 1993; Quinlan & Hadley, 1993; Lighton & Berrigan, 1995; Lighton & Garrigan, 1995; Bosch *et al.*, 2000; Chown & Holter, 2000). In sharp contrast, this broad scale analysis suggests that this is indeed the case. One explanation for these contrary findings might be that reported metabolic and water loss rates vary in a consistent manner, between the xeric and mesic species, because of the measuring technique adopted (e.g. Machin *et al.*, 1991; Lighton & Fielden, 1995). Given that the majority of the data used in this study for a test of the ZM were collected using non flow-through methods, this explanation is not likely. Rather, it is suspected that there have been insufficient studies using flow through methods across a wide range of closely related species, occupying different envioronments, to provide a careful analysis of the proportional contributions of respiratory and cuticular transpiration to total water loss. Thus, despite the considerable literature that is available on insect water balance, there is obviously still much to be learnt about the relative contribution of the different water loss avenues in insects.

Second, the broad scale analyses undertaken here suggest that metabolic rate does not differ significantly between mesic and xeric species. In contrast, finer scale studies have shown that this is the case (Edney, 1977; Juliano, 1986; Lighton &

Bartholomew, 1988; Hoffmann & Parsons, 1991; Cushman *et al.*, 1993; Wasserthal 1996; Chown, *at al.*, 1997). Undoubtedly, the relatively small number of species for which data on both metabolic rate and desiccation rate are available mean that this analysis might not have the resolution to extract such variation. Moreover, a study by Davis *et al.* (2000) has demonstrated that inferring the direction of change in metabolic rate across species from mesic and xeric environments is likely to be problematic. Perhaps the most appropriate solution to this particular conundrum is to base further investigations on laboratory selection (Gibbs, 1999), though even here results might be difficult to interpret (Djawdan *et al.*, 1997; Williams *et al.*, 1997; Williams & Bradley, 1998; Bradley *et al.*, 1999). Nonetheless, it is clear that studies of desiccation resistance in insects need no longer be confined to extreme environments where the effects of the environment can be readily identified (Bartholomew, 1987). Rather, the capabilities of the instrumentation that is now available mean that a selection of species, from environments with conditions appropriate for crucial tests (e.g. insects from cold vs warm dry environments at similar altitudes, see Davis *et al.*, 2000), might readily be made.

Finally, the test of the ZM used a selection of mostly flightless species, but without considering higher taxonomic group. The results of the nested analysis of variance indicated that a substantial portion of the variance is partitioned above the species level (see also Chown *et al.*, 1999). While this finding provides some support for Zachariassen (1996) model, given that the ZM predicts consistent differences between higher taxa, this higher level variation also suggests that the test of the ZM model may be confounded to some extent by phylogenetic non-independence (see above). At present, there are no means of determining the likely effect of this hierarchical variation, though I think it is important that this should be done. In other words, there is substantial scope for careful, combined studies of metabolic rate and the avenues of water loss in terrestrial insects across a range of higher taxa.

In conclusion, this study provides quantitative, broad scale support for much of what has previously been assumed regarding desiccation resistance in insects. However, it also shows that additional studies are required to place the field on a firm, predictive footing, especially regarding the relative contributions of respiratory and cuticular transpiration to overall water loss. There is also a need for a careful investigation of the Zachariassen model, using laboratory selection and flow through techniques, and an appropriate selection of species from a range of higher taxa, is one useful route that might be followed.

References

Addo-Bediako, A., Chown, S.L., Gaston, K.J. 2000. Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London B* **267**, 739-745.

Ahearn, G.A. 1970. The control of water loss in desert tenebrionid beetles. *Journal of Experimental Biology* **53**, 573-595.

Ahearn, G.A. & Hadley, N.F. 1969. The effects of temperature and humidity on water loss in two desert tenebrionid beetles, *Eleodes armata* and *Cryptoglossa verrucosa*. *Comparative Biochemistry and Physiology* **30**, 739-749.

Arlian, L.G. & Veselica, M.M. 1979. Water balance in insects and mites. *Comparative Biochemistry and Physiology* **64A**, 191-200.

Bale, J.S. 1993. Classes of insect cold hardiness. *Functional Ecology* 7, 751-753.

Bartholomew, G.A. 1987. Interspecific comparison as a tool for ecological physiologists. In: Feder, M.E., Bennet, A.F., Burggren, W., Huey, R.B. (Eds.) *New Directions in Ecological Physiology*. Cambridge University Press, Cambridge, pp. 11-37.

Baum, D.A. & Larson, A. 1991. Adaptation reviewed: a phylogenetic methodology for studying macroevolution. *Systematic Zoology* **40**, 1-18.

Bosch, M., Chown, S.L. & Scholtz, C.H. 2000. Discontinuous gas exchange and water loss in the keratin beetle *Omorgus radula*: further evidence against the water conservation hypothesis? *Physiological Entomology* **24**, 309-314.

Bradley, T.J., Williams, A.E. & Rose, M.R. 1999. Physiological responses to selection for desiccation resistance in *Drosophila melanogaster*. *American Zoologist* **39**, 337-345.

Bryant, S.R., Thomas, C.D., & Bale, J.S. 1997. Nettle-feeding nymphalid butterflies: temperature, development and distribution. *Ecological Entomology* **22**, 390-398.

Casti, J.L. 1991. *Searching for certainty. What science can know about the future*. Abacus, London.

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

Chown, S.L. & Clark, A. 2000. Stress and the geographic distribution of marine and terrestrial animals. In: Story, K.B. (Eds.) *Cell and Molecular Responses to Stress*. Elsevier, Amsterdam, pp. (in press).

Chown, S.L. & Gaston, K.J. 1999. Exploring links between physiology and ecology at macro scales: the role of respiratory metabolism in insects. *Biological Reviews* **74**, 87-120.

Chown, S.L. & Holter, P. 2000. Discontinuous gas exchange cycles in *Aphodius fossor* (Scarabaeidae): a test of hypothesis concerning origins and mechanisms. *Journal of Experimental Biology* **203**, 397-403.

Chown, S.L., Le Lagadec, M.D. & Scholtz, C.H. 1999. Partitioning variance in a physiological trait: desiccation resistance in keratin beetles (Coleoptera: Trogidae). *Functional Ecology* **13**, 838-844.

Chown, S.L., Pistorius, P.A. & Scholtz, C.H. 1998. Morphological correlates of flightlessness in southern African Scarabaeidae (Coleoptera: Scarabaeidae): testing a condition of the water conservation hypothesis. *Canadian Journal of Zoology* **76**, 1123-1133.

Chown, S.L., Scholtz, C.H., Klok, C.J., Joubert, F.J. & Coles, K. 1995. Ecophysiology, range contraction and survival of a geographically restricted African dung beetle (Coleoptera: Scarabaeidae). *Functional Ecology* **9**, 30-39.

Chown, S.L., Van der Merwe, M. & Smith, V.R. 1997. The influence of habitat and altitude on oxygen uptake in sub-Antarctic weevils. *Physiological Zoology* **70**, 116-124.

Cloudsley-Thompson, J.L. 1964. On the function of the sub-elytral cavity in desert tenebrionidae (Col.). *Entomologist's Monthly Magazine* **100**, 148-151.

Coddington, J.A. 1988. Cladistic tests of adaptational hypothesis. *Cladistics* **4**, 3-22.

Collins, M.S. & Richards, A.G. 1966. Studies on water relations in north American termites. II. Water loss and cuticular structures in eastern species of the Kalotermitidae (Isoptera). *Ecology* **47**, 328-331.

Cushman, J.H., Lawton, J.H. & Manly, B.F.J. 1993. Latitudinal patterns in European ant assemblages: variation in species richness and body size. *Oecologia* **95**, 30-37.

Davidson, J.K. 1990. Non-parallel geographic patterns for tolerance to cold and desiccation in *Drosophila melanogaster* and *D. simulans*. *Australian Journal of Zoology* **38**, 155-161.

Davis, A.J., Jenkinson, L.S., Lawton, J.H., Shorrocks, B. & Wood, S. 1998. Making mistakes when predicting shifts in species range in response to global warming. *Nature* **391**, 783-786.

Davis, A.L.V., Chown, S.L., McGeoch, M.A. & Scholtz, C.H. 2000. A comparative analysis of metabolic rate in six *Scarabaeus* species (Coleoptera: Scarabaeidae) from southern Africa: further caveats when inferring adaptation. *Journal of Insect Physiology* **46**, 553-562.

Davis, A.L.V., Chown, S.L. & Scholtz, C.H. 1999. Discontinuous gas-exchange cycles in *Scarabaeus* dung beetles (Coleoptera: Scarabaeidae): mass-scaling and temperature dependence. *Physiological and Biochemical Zoology* **72**, 555-565.

Djawdan, M., rose, M.E. & Bradley, T.J. 1997. does selection for stress resistance lower metabolic rate? *Ecology* **78**, 828-837.

Draney, M.L. 1993. The subelytral cavity of desert tenebrionids. *Florida Entomologist* **76**, 539-548.

Eckstrand, I.A. & Richardson, R.H. 1981. Relationships between water balance propertire and habitat characteristics in the sibling Hawaiian Drosophilids, *D. mimica* and *D. Kambysellisi*. *Oecologia* **50**, 337-341.

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

Edney, E.B. 1982. The truth about saturation deficiency- an historical perspective. *Journal of Experimental Zoology* **222**, 205-214.

Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist* **125**, 1-15.

Freckleton, R.P. 2000.Phylogenetic tests of ecological and evolutionary hypothesis: checking for phylogenetic independence. *Functional Ecology* **14**, 129-134.

Gelman, N., Machin, J. & Kestler, P. 1988. The nature of the driving forces for passive water transport through arthropods cuticle. *Journal of Thermal Biology* **13**, 157-762.

Gereben, B.-A. 1995. Co-occurrence and microhabitat distribution of six *Nebria* species (Coleoptera: Carabidae) in an alpine gracier retreat zone in the Alps, Austria. *Artic and Alpine Researh* **27**, 371-379.

Gibbs, A. Mousseaus, T,A,, Crowe, J.H. Genetic and acclamatory variation in biophysical properties of insect cuticle lipids. *Proceedings of the National Academy of Sciences of the USA* **88**, 7257-7260.

Gibbs, A.G. 1998. Water-proofing properties of cuticular lipids. *American Zoologist* **38**, 471-482.

Gibbs, A.G. 1999. Laboratory selection for the comparative physiologist. *Journal of Experimental Biology* **202**, 2709-2718.

Gilby, A.R. 1980. Transpiration, temperature and lipids in insect cuticle. *Advances in Insect Physiology* **15**, 1-33.

Hadley, N.F. 1994. *Water relations of terrestrial arthropods*. Academic Press, San Diego.

Hadley, N.F. & Massion, D.D. 1985. Oxygen consumption, water loss and cuticular lipids of high and low elevation populations of the grasshopper *Aeropedellus clavatus* (Orthoptera: Acrididae). *Comparative Biochemistry and Physiology* **80A**, 307-311.

Hadley, N.F. & Quinlan, M.C. 1993. Discontinuous carbon dioxide release in the eastern lubber grasshopper *Romalea guttata* and its effect on respiratory transpiration. *Journal of Experimental Biology* **177**, 169-180.

Harvey, P.H. & Pagel, M.D. 1991. *Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.

Hesse, A.J. 1938. Some adaptive responses of insect life to semi-arid conditions in South Africa. *South African Journal of Science* **35**, 69-92.

Hawitson, B.C. 1997. A methodology for developing regional climate change scenarios from general circulation models. *Water Research Commision Report, South Africa* 594/1/97.

Hochberg, M.E. & Ives, A.R. 1999. Can natural enemies enforce geographical range limits? *Ecography* **222**, 268-276.

Hoffmann, A. & Parsons, P.A. 1991. *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford.

Hood, W.G. & Tschinkel, W.R. 1990. Desiccation resistance in aboreal terrestrial ants. *Physiological Entomology* **15**, 23-35.

Jeffree, C.E. & Jeffree, E.P. 1996. Redistribution of the potential geographical ranges of Mistletoe and Colorado beetle in Europe in response to the temperature component of climate change. *Functional Ecology* **10**, 562-577.

Jeffree, E.P. & Jeffree, C.E. 1994. Temperature and biogeographical distributions of species. *Functional Ecology* **8**, 640-650.

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

Juliano, S.A. 1986. Resistance to desiccation and starvation of two species of Brachinus (Coleoptera: Carabidae) from southeastern Arizona. *Canadian Journal of Zoology* **64**, 73-80.

Kerr, J. & Packer, L. 1998. The impact of climate change on mammal diversity in Canada. *Environmental Monitoring Assessment* **49**, 263-270.

Klok, C.J. & Chown, S.L. 1998. Interactions between desiccation resistance, host-plant contact and the thermal biology of a leaf-dwelling sub-Antarctic caterpillar, *Embryonopsis halticella* (Lepidoptera: Yponomeutidae). *Journal of Insect Physiology* **44**, 615-628.

Le Lagadec, M.D. 1997. Geographical variation in desiccation tolerance in southern African Trogidae (Coleoptera). PhD thesis, University of Pretoria, Pretoria.

Le Lagadec, M.D., Chown, S.L. & Scholtz, C.H. 1998. Desiccation resistance and water balance in southern African keratin beetles (Coleoptera: Trogidae): the influence of body size, habitat and phylogeny. *Journal of Comparative Physiology B* **168**, 112-122.

Lewis Smith, R.I. & Walton, D.W. H. 1975. South Georgia, Sub-Antarctic. *Ecological Bulletin* **20**, 399-423.

Lighton, J.R.B. 1991. Measurements on insects. In: Payne, P.A. (Eds.) *Concise Encyclopedia on Biological and Biomedical Measurement Syetems*. Pergamon Press, Oxford, pp 201-208.

Lighton, J.R.B. 1996. Discontinuous gas exchange in insects. *Annual Review of Entomology* **41**, 309-324.

Lighton, J.R.B. 1998. Notes from underground: towards ultimate hypotheses of cyclic, discontinuous gas-exchange in tracheate arthropods. *American Zoologist* **38**, 483-491.

Lighton, J.R.B. & Bartholomew, G.A. 1988. Standard energy metabolism of a desert harvester ant, *Pogonomyrmex rugosus*: effects of temperature, body mass, group size, and humidity. *Proceedings of National Academy of Sciences of USA* **85**, 4765-4769.

Lighton, J.R.B. & Berrigan, D. 1995. Questioning paradigms: caste-specific ventilation in harvester ants, *Messor pergandei* and *M. julianus* (Hymenoptera: Formicidae). *Journal of Experimental Biology* **198**, 521-530.

Lighton, J.R.B. & Fielden, L.J. 1995. Mass scaling of standard metabolism in ticks: a valid case of low metabolic rates in sit- and wait- strategists. *Physiological Zoology* **68**, 43-62.

Lighton, J.R.B. & Garrigan, D. 1995. Ant breathing: testing regulation and mechanism hypotheses with hypoxia. *Journal of Experimental Biology* **198**, 1613-1620.

Lighton, J.R.B., Quinlan, M.C., & Feener, D.H. 1994. Is bigger better? Water balance in the polymorphic desert harvester ant *Messor pergandei*. *Physiological Entomology* **19**, 325-334.

Machin, J., Kestler, P. & Lampert, G.J. 1991. Simultaneous measurements of spiracular and cuticular water loses in *Periplaneta americana*: implications for whole-animal mass loss studies. *Journal of Experimental Biology* **161**, 439-453.

Marsh, A.C. 1988. Activity patterns of some Namib desert ants. *Journal of Arid Environments* **14**, 61-73.

McCullagh, . & Nelder, J.A. 1989. *Generalized Linear Models*. Chapman and Hall, London.

Monteith, J.L. & Campbell, G.S. 1980. Diffusion of water vapour through integuments-potential confusion. *Journal of Thermal Biology* **5**, 7-9.

Nagel, E. 1961. *The Structure of Science. Problems in the Logic of Scientific Explanation*. Routledge & Kegan Paul, London.

Naidu, S.G. & Hattingh, j. 1988. Water balance and osmoregulation in *Physadesmia globosa*, a diurnal tenebrionid beetle from the Namib desert. *Journal of Insect Physiology* **34**, 911-917.

Nicolson, S.W. 1980. Water balance and osmoregulation in *Onymacris plana*, a tenebrionid beetle from the Namib desert. *Journal of Insect Physiology* **26**, 315-320.

Nicolson, S.W., Louw, G.N. & Edney, E,B. 1984. Use of a ventilated capsule and tritiated water to measure evaporative water losses in a tenebrionid beetle. *Journal of Experimental Biology* **108**, 477-481.

North, R.d. 1991. Transpiration and humidity preferences in a temperate wood ant *Formica rufa* L. *Journal of Insect Physiology* **37**, 279-286.

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J.K., Thomas, C.D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W.J., Thomas, J.A. & Warren, M. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature* **399**, 579-583.

Peters, R.H. 1983. *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge.

Price, T. 1997. Correlated evolution and independent contrasts. *Philosophical Transactions of the Royal Society, London B* **352**, 519-529.

Quinlan, M.C. & Hadley, N.F. 1993. Gas exchange, ventilatory patterns, and water loss in two lubber grasshoppers: quantifying cuticular and respiratory transpiration. *Physiological Zoology* **66**, 628-642.

Reinhold, K. 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology* **13**, 217-224.

Ricklefs, R.E. & Starck, J.M. 1996. Applications of phylogenetically independent contrasts: a mixed progress report. *Oikos* **77**, 167-172.

Robinson, T., Rogers, D. & Williams, B. 1997a. Mapping tsetse habitat suitability in the common fly belt of southern Africa using multivariate analysis of climate and remotely sensed vegetation data. *Medical and Veterinary Entomology* **11**, 235-245.

Robinson, T., Rogers, D. & Williams, B. 1997b. Univariate analysis of tsetse habitat in the common fly belt of southern Africa using climate and remotely sensed vegetation data. *Medical and Veterinary Entomology* **11**, 223-234.

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

Salin, C., Vernon, P. & Vannier, G. 1999. Effects of humidity on transpiration in adults of the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). *Journal of Experimental Physiology* **45**, 907-914.

Schmidt-Nielsen, K. 1984. *Scaling. Why is Animal Size so Important?* Cambridge University Press, Cambridge.

Schneider, S.H. 1992. The climatic response to Greenhouse gases. *Advances in Ecological Research* **22**, 1-32.

Scholtz, C.H. 1981. Aptery in *Trox* (Coleoptera: Trogidae): morphological changes and their relationship to habitat. *Journal of the Entomological Society of Southern Africa* **44**, 83-87.

Schulze, B.R. 1971. The climate of Marion Island. In: van Zinderen Bakker, E.M., Winterbottom, J.M., Dyer, R.A. (Eds.) *Marion and Prince Edward Islands. Report on the South African biological expedition 1965-1966*. A.A. Balkema, Cape Town, pp. 16-31.

Sokal, R.R. & Rohlf, F.J. 1995. *Biometry: The Principles of Statistics in Biological Research*. W.H. Freeman, New York.

Sømme, L. 1995. *Invertebrates in Hot and Cold Arid Environments*. Springer-Verlag, Berlin.

Toolson, E.C. 1978. Diffusion of water through the arthropod cuticle: thermodynamic consideration of the transition phenomenon. *Journal of Thermal Biology* **3**, 69-73.

Toolson, E.C. 1980. Thermodynamic and kinetic aspects of water flux through arthropod cuticle. *Journal of Thermal Biology* **5**, 1-6.

Ward, D. & Seely, M.K. 1996. Adaptation and constraint in the evolution of the physiology and behavior of the Namib desert tenebrionid beetle genus *Onymacris*. *Evolution* **50**, 1231-1240.

Wasserthal, L.T. 1996. Interaction of circulation and tracheal ventilation in holometabolous insects. *Advances in Insect Physiology 26*, 297-351.

Wharton, G.W. 1985. Water balance of insects. In: Kerkut, G.A., Gilbert, L. I. (Eds.) *Comparative Insect Biochemistry, Physiology and Pharmacology*. Pergamon Press, Oxford, pp. 565-601.

Williams, A.E. & Bradley, T.J. 1998. The effect of respiratory pattern on water loss in desiccation-resistant *Drosophila melanogaster*. *Journal of Experimental Biology* **201**, 2953-2959.

Williams, A.E., Rose, M.R. & Bradley, T.J. 1997. CO_2 release patterns in *Drosophila melanogaster*: the effect of selection for desiccation resistance. *Journal of Experimental Biology* **200**, 615-624.

Williams, P.H. 1998. *Worldmap IV 4.19.17*. Privately published, London.

Zachariassen, K.E. 1991. Routes of transpiratory water loss in a dry-habitat tenebrionid beetle. *Journal of Experimental Biology* **157**, 425-437.

Zachariassen, K.E. 1996. The water conserving physiological compromise of desert insects. *European Journal of Entomology* **93**, 359-367.

Zachariassen, K.E., Andersen, J., Maloiy, G.M.O. & Kimau, J.M.Z. 1987. Transpiratory water loss and metabolism of beetles from arid areas in East Africa. *Comparative Biochemistry and Physiology* **86A**, 403-408.

Zachariassen, K.E., Andersen, J., Kimau, J.M.Z. & Maloiy, G.M.O. 1988. Water loss in insects from arid and humid habitats in East Africa. *Acta Entomologica Bohemoslovaca* **85**, 81-93.

Zachariassen, K.E. & Maloiy, G.M.O. 1989. Water balance of beetles as an indicator of environmental humidity. *Fauna Norvegica B* **36**, 27-31.

Chapter 4

Latitudinal variation and the temperature dependence of insect development

4.1 Introduction

Temperature is considered one of the most significant abiotic factors that affects the growth and development of ectotherms. Indeed, not only is there a direct relationship between development rate and temperature in ectotherms, but temperature requirements during development vary both between species from the same area and between species from different geographical areas (Campbell *et al.*, 1974). Thus, most ectotherms can only develop within a certain range of temperatures and often this range is narrow. Since the rate of development depends directly on temperature, it might be expected that the lower environmental temperature and/or shorter developing (growing) season of high latitude environments would slow the rate of growth/development in these regions. However, contrary to this expectation, some studies have shown that high latitude species often develop faster than those from low latitudes within a certain temperature range (e.g. Conver & Present, 1990).

The same sorts of variation have been attributed to and found in insect ecotherms (e.g. Sømme & Block, 1991). Two hypotheses have been proposed to explain latitudinal variation in ectotherm development. In the first case, it is thought that individuals at high latitudes (or those that occur where environmental temperatures are often low) compensate for the short growing season by developing rapidly during this relatively warm period. That is, the individuals of species from high latitudes grow more rapidly than their low latitude counterparts at approximately the same temperatures (see Gotthard *et al.*, 2000). This selection for rapid development is also known as 'countergradient variation' (Levins, 1969; Conover & Present, 1990), and is known to be associated with a variety of both physiological and ecological costs (Werner & Anholt, 1993; Gotthard *et al.*, 1994).

The second hypothesis, discussed most recently by Levinton (1983) and also by Trudgill & Perry (1994) (see also Trudgill, 1995), suggests that high latitude species develop more rapidly at cold temperatures than their tropical counterparts, and *vice versa*. In other words, the rate-development curves of tropical and temperate species should intersect, such that rates in the tropical species are always highest at high temperatures and those of temperate species highest at low temperatures. In many cases this pattern might also be termed latitudinal compensation. Such latitudinal compensation has also been demonstrated in other physiological processes such as respiratory metabolism (see Scholander *et al.*, 1953; Bullock, 1955; Block & Young, 1978; Cossins & Bowler, 1987; Berrigan & Partridge, 1997; Addo-Bediako *et al.*, submitted), though it remains polemical (Clark, 1991, 1993; Chown & Gaston, 1999).

Each of these major hypotheses implies certain relationships between development rate, lower development threshold (LDT) (temperature at which development ceases), the sum of effective temperatures (SET) (the amount of heat needed for completion of a development stage, expressed as day degrees), and latitude. If the latitudinal compensation hypothesis is correct, then development rate should vary with trial temperature, but once this is taken into account, environmental temperature and development rate should be negatively related. In the case of the countergradient variation hypothesis, mean environmental temperature and development rate should show no relationship at all. Likewise, for the latitudinal compensation hypothesis to be supported, there should be a positive relationship between LDT and environmental temperature, and a negative relationship between LDT and SET (Honêk & Kocourek, 1990). It is only this relationship that leads to greater development rates at low temperatures in temperate compared to tropical species and *vice versa*. If the countergradient hypothesis is correct then there should either be no relationship between LDT and environmental temperature, and a non-significant relationship between LDT and SET, or a positive relationship between LDT and environmental temperature and a positive relationship between LDT and SET. Furthermore, if the countergradient hypothesis is correct then SET should show a positive relationship with environmental temperature, while if the latitudinal compensation hypothesis is correct the relationship should be negative.

To test these competing hypotheses, a synthetic, quantitative analysis of latitudinal patterns of variation in temperature dependence of development in insects, based on the published literature, is used.

4.2 Materials and methods

Published papers on temperature dependence of development in insects were examined as far back as 1900, with major emphasis on the last 50 years. From each of these studies, the following information was obtained: trial temperature, duration of development (in days), geographic location (expressed in the form of latitude and longitude) and stage of development. Only data from studies where total development (from egg to adult) was reported were used in the analysis. Studies were excluded if only the lower development threshold (LDT) and the sum of effective temperatures (SET) were provided, rather than the rate of development or duration of development at each trial temperature. Where a species was encountered twice or more, then the record from the geographic location that was least represented in the database was selected. Thus, the primary database included data on 216 species from 72 Families and 9 Orders, where development had been investigated at three or more temperatures. For each of these species, environmental temperature of their habitat was determined by assigning a value, based on geographic position, from the half-degree mean annual temperature database (1961-1990) provided by the Intergovernmental Panel on Climate Change Data Distribution Centre (http://ipcc-ddc.cru.uea.ac.uk/ipcc_ddc/index.html). The influence of trial temperature and environmental temperature on development rate were then investigated using a generalised linear model (McCullagh & Nelder, 1989).

LDT and SET were calculated from data on duration of development under constant temperatures, using only those studies where development was examined at three or more trial temperatures. Calculation of LDT and SET are well documented (e.g. Campbell *et al.*, 1974; Lamb, 1992). For the most part, it can be assumed that development rate, R(T), increases linearly with trial temperature, T, and is given by the equation $R(T) = a + bT$, where a is the intercept and b is the slope, and these are estimated by linear regression. The lower development threshold is calculated as $-a/b$, and sum of effective temperatures as $1/b$.

Finally, because it has been argued that development rate varies considerably between different taxa (i.e. is influenced by phylogenetic propinquity) (Honêk, 1999), the taxonomic level at which most variance in rate of development was partitioned was investigated using a nested analysis of variance with the Satterthwaite approximation for unequal sample sizes (Sokal & Rohlf, 1995). Variance was partitioned between the species (including the error term), generic, familial, and ordinal levels using only those studies conducted at or close to 25°C.

4.3 Results

The nested analysis of variance indicated that most variance was partitioned at the family level (50.70%, $p<0.0001$), while the order, genus, and species levels accounted for a lower, though not insubstantial portion of this variance (13.80%, $p>0.05$; 17.00%, $p<0.01$; 18.50% respectively). Because of the poor geographic coverage of data at the family level, but the indication that phylogenetic relatedness accounts for an important proportion of the variation in development rate, all subsequent analyses were also undertaken separately on the five major insect orders (Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera). Likewise, initial inspection of the data (e.g. Fig. 4.1 development rate and latitude) indicated that tropical species were underrepresented in the database. Thus all analyses were undertaken including these few species, and excluding all records from between 25°S and 25°N, except in the case of the Diptera and Hymenoptera, where no tropical species were included.

For the full set of data, both trial temperature and environmental temperature entered the generalized linear model significantly, with trial temperature having the expected positive effect, and environmental showing a rather surprising inverse relationship with development rate (Table 4.1 all species and temperate species and all orders). However, this appeared to be due largely to the few, tropical species, which tend to form a biased subset of the available species in any case (see Discussion). Removing these species indicated no relationship between environmental temperature and development rate (Table 4.1). This pattern was not consistent between Orders, with the Diptera, Hymenoptera and Lepidoptera supporting the general pattern, and the Coleoptera and Hemiptera providing some evidence that the negative

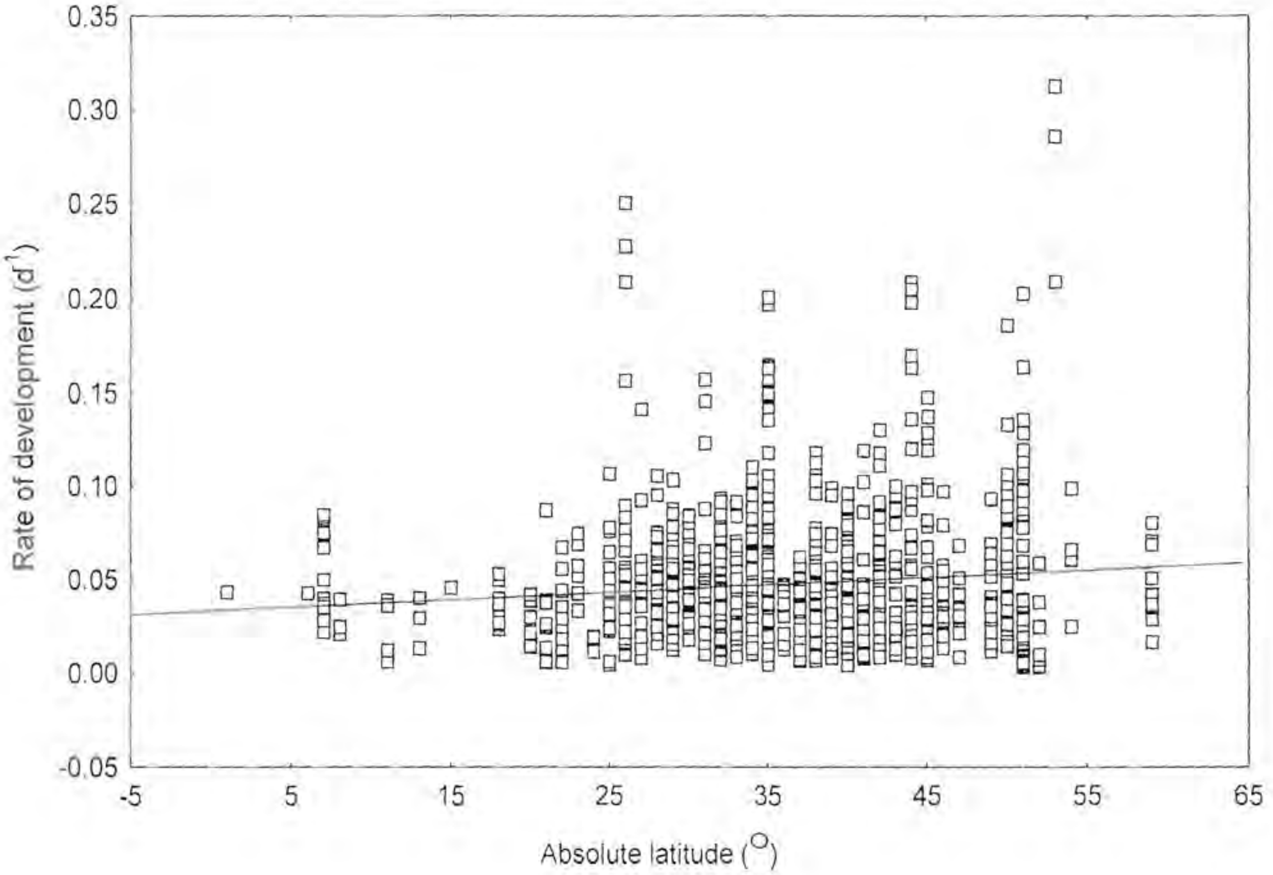

Figure. 4.1. Relationship between rate of development and geographical latitude (°N and °S) of species site of origin.

relationship with latitude remains even when tropical species are excluded (Table 4.1). The relationship between LDT and environmental temperature was generally significant and positive, with the exception of that for the Diptera and Hymenoptera (whether or not the tropical species were included (Table 4.2).

Table 4.1. Generalized linear model of the explanatory variable on $\log_{10}$ development rate.

Explanatory variables (Goodness of fit)	Parameter estimate	df (df)	Type III log-likelihood (Deviance)	Chi-square (Deviance/df)	p
All species					
Trial temperature	0.027	1	-229.99	355.02	0.0001
Environmental temperature	-0.002	1	-57.46	9.95	0.0016
		(1033)	(67.13)	(0.06)	
All temperate species					
Trial temperature	0.027	1	-222.47	321.54	0.0001
Environmental temperature	-0.001	1	-62.62	1.83	0.18
		(965)	(64.38)	(0.07)	
Coleoptera					
Trial temperature	0.03	1	-50.21	137.06	0.0001
Environmental temperature	-0.01	1	-0.69	38.02	0.0001
		(243)	(12.41)	(0.05)	
Coleoptera (temperate)					
Trial temperature	0.03	1	-49.37	131.43	0.0001
Environmental temperature	-0.012	1	-3.73	40.15	0.0001

		(233)	(12.03)	(0.05)	
Diptera					
Trial temperature	0.03	1	-8.18	114.43	0.0001
Environmental temperature	0.003	1	47.32	3.41	0.06
		(133)	(3.87)	(0.03)	
Hemiptera					
Trial temperature	0.02	1	-41.99	54.69	0.0001
Environmental temperature	-0.007	1	-24.72	20.15	0.0001
		(198)	(13.61)	(0.07)	
Hemiptera (temperate)					
Trial temperature	0.03	1	-41.76	41.92	0.0001
Environmental temperature	-0.011	1	-32.94	24.27	0.0001
		(169)	(12.83)	(0.08)	
Hymenoptera					
Trial temperature	0.03	1	-16.94	183.81	0.0001
Environmental temperature	0.001	1	74.79	0.36	0.54
		(247)	(8.035)	(0.03)	
Lepidoptera					
Trial temperature	0.03	1	-24.38	123.01	0.0001
Environmental temperature	0.0001	1	37.12	0.01	0.9
		(149)	(5.46)	(0.04)	
Lepidoptera (temperate)					
Trial temperature	0.034	1	-20.66	102.19	0.0001
Environmental temperature	0.002	1	29.65	1.58	0.21
		(129)	(4.87)	(0.04)	

Table 4.2. Generalized linear model of environmental temperature on lower development threshold (LDT).

Explanatory variables (Goodness of fit)	Parameter estimate	df (df)	Type III log-likelihood (Deviance)	Chi-square (Deviance/df)	p
All species	0.115	1	-597.69	20.7	0.0001
		(214)	(2909.63)	(13.60)	
All temperate species	0.09	1	-551.29	9.06	0.0024
		(199)	(2713.90)	(13.64)	
Coleoptera	0.018	1	-124.17	15.76	.0001
		(46)	(357.34)	(7.80)	
Coleoptera (temperate)	0.168	1	-116.03	10.21	0.001
		(44)	(334.76)	(7.61)	
Diptera	0.011	1	-76.23	2.28	0.14
		(26)	(351.37)	(13.50)	
Hemiptera	0.19	1	-127.5	16.25	0.0001
		(43)	(530.80)	(12.30)	
Hemiptera (temperate)	0.18	1	-106.78	8.32	0.004
		(36)	(493.05)	(13.70)	

Hymenoptera	0.02	1	-156.0	0.17	0.68
		(54)	(859.28)	(15.91)	
Lepidoptera	0.10	1	-65.02	8.27	0.004
		(27)	(113.10)	(4.2)	
Lepidoptera (temperate)	0.09	1	-54.63	4.003	0.045
		(23)	(98.66)	(4.29)	

However, SET and environmental temperature showed no significant relationship, except in the case of the Lepidoptera. Likewise, the relationship between LDT and SET was not significant in most cases ($0.327 < p < 0.929$), except for the Diptera and Lepidoptera (with and without tropical species), where the relationship was negative (Table 4.3).

Table 4.3. Generalized linear model of sum of effective temperatures (SET) on lower development threshold (LDT).

Explanatory variables (Goodness of fit)	Parameter estimate	df (df)	Type III log-likelihood (Deviance)	Chi-square (Deviance/df)	p
All species	-0.001	1	-597.69	0.96	0.33
		(214)	(3188.05)	(14.9)	

All temperate species	-0.001	1	-551.29	0.6	0.44
		(199)	(2829.57)	(14.22)	
Coleoptera	-0.0002	1	-124.17	0.008	0.93
		(46)	(496.11)	(10.79)	
Coleoptera (temperate)	0.0007	1	-116.03	0.11	0.74
		(44)	(416.96)	(9.48)	
Diptera	-0.12	1	-76.23	12.56	0.0004
		(26)	(242.39)	(9.30)	
Hemiptera	-0.0003	1	-127.5	0.009	0.92
		(43)	(761.46)	(17.71)	
Hemiptera (temperate)	0.002	1	-106.78	0.35	0.56
		(36)	(608.15)	(16.89)	
Hymenoptera	-0.007	1	-156.0	6.54	0.01
		(54)	(766.77)	(14.20)	
Lepidoptera	-0.004	1	-65.02	2.82	0.09
		(27)	(136.45)	(5.05)	
Lepidoptera(temperate)	-003	1	-54.63	1.55	0.21
		(23)	(108.84)	(4.73)	

4.4 Discussion

Prior to any discussion of the results obtained in this analysis, there are several important points that should be raised. First, the sample size in this study was not large (216) and it is clear that tropical species are underrepresented in the database (Fig. 4.1). Furthermore, much of the variance in development rate tends to be partitioned at higher taxonomic levels, making interpretation of the data more difficult, especially because sample sizes are smaller within higher taxa (e.g. Coleoptera 48/45 (all/extra-tropical); Diptera 28/28; Hemiptera 45/38; Hymenoptera 55/55; Lepidoptera 30/26). Indeed, (Honêk, 1999) has shown in a similar study that both taxonomic group and body size have a considerable influence on the rate of development recorded for any given insect species. While some attempt has been made to correct for the former here, many of the studies from which data were obtained did not report body size, making inclusion of the latter problematic. These potentially confounding factors should be borne in mind during the discussion that follows.

Returning to the two hypotheses regarding latitudinal (or more correctly environmental temperature related) variation in development rate, it is immediately apparent that neither hypothesis enjoys firm support, at least not with the data at hand (Table 4.4).

Table 4.4. Effect of environmental temperature on the rate of development, LDT and SET and the relationship between LDT and SET. (+ denotes positive significant relationship, - denotes negative significant relationship and ns denotes no significant relationship).

Group	Development rate	LDT	SET	LDT/SET
All species	-	+	ns	ns
Non-tropical species	ns	+	ns	ns
Coleoptera	-	+	ns	ns
Non-tropical	-	+	ns	ns
Diptera	ns	ns	ns	-
Hemiptera	-	+	ns	ns
Non-tropical	-	+	ns	ns
Hymenoptera	ns	ns	ns	-
Lepidoptera	ns	+	-	ns
Non-tropical	ns	+	-	ns

In the case of development rate, there is no environment-related variation, supporting the countergradient variation hypothesis, at least so far as all of the species and the Diptera, Hymeoptera and Lepidoptera are concerned. In the Coleoptera and Hemiptera it appears that the latitudinal compensation hypothesis does enjoy support. However, further tests do not bear out this suggestion, especially because there tends to be no relationship between SET and environmental temperature, and no relationship between LDT and SET. Even where there is variation between Orders, this variation is not consistent such that it provides unequivocal support for either of the hypotheses. Rather what appears to be the most general pattern is a decline in LDT with environmental temperature, but no variation in SET and no relationship between SET and LDT. In other words, development rate (and its temperature sensitivity) does not vary with environmental temperature, but the lower developmental threshold does. Thus, temperate species can develop at lower temperatures than tropical ones, but appear to do so at the same rate. In one sense this can be regarded a form of latitudinal compensation, equivalent to translation of the rate-temperature curve often found in acclimation experiments (Cossins & Bowler, 1987), and often attributed to alterations in metabolic rate of temperate vs. tropical species (see Chown & Gason, 1999).

While it may be tempting to adopt this finding as an overall conclusion, there are several points that preclude this. Most notably, there is a great deal of variability about the relationships, probably a function of the considerable variety in body size and diet of the species that have been included. Honêk (1996a, 1999) has demonstrated that both diet and body size have a significant effect on development in insects. Controlling for this variation, he concluded that LDT varies inversely with latitude, that there is a significant negative relationship between LDT and SET, and that there is consequently considerable support for the latitudinal compensation hypothesis (as formulated by Levinton, 1983; Trudgill, 1995). However, it should be noted that Honêk (Honêk, 1996a, 1999) was unable to demonstrate these relationships for the full development of insects (egg to adult), but could only do so for certain stages. Thus, it appears that the findings of the current study are broadly in agreement with those of Honêk (1996a, b). Be that as it may, it is clear that body size, diet and phylogeny will always act to confound analyses such as the current one, and this is a point that has broader implications than simply an investigation of the extent of latitudinal compensation (see below).

Furthermore, recent work by (Gotthard *et al.*, 2000) has shown that the time constraint faced by a species may also influence considerably its development rate. Thus the season during which a study was undertaken might have a considerable influence on the results obtained. In some circumstances there is countergradient variation, whereas in others this might not be the case. Work by Bryant *et al.* (1998) has also indicated that behavioural thermoregulation can make a large difference to degree-day accumulation in insects, though this is rarely considered in laboratory studies or calculations of SET. Given that both Chown (1997) and Chown and Gaston (1999) have suggested that such behavioural thermoregulation might affect the likelihood of latitudinal compensation in metabolic rate (see Chapter 5), the importance of behavioural thermoregulation, and the opportunities for insects to do so, also deserves exploration in the context of large scale patterns in insect development rate.

In sum, it seems that at this point the evidence for or against either of the two primary hypotheses is largely inconclusive. This raises two important issues. First, despite a reasonably large literature on insect development (see Honêk, 1996b) and the Appendix to this thesis) there is obviously scope for many more studies, particularly those that report body size and diet of the species involved. In particular, a great deal of work needs to be done in the southern hemisphere, where comparatively few investigations have been undertaken (see Chapter 6). Second, hypotheses regarding geographic variation in insect development rate and voltinism, and their evolutionary implications are probably not well supported.

Rohde (1992, 1997) proposed that higher species richness in the tropics is a consequence of rapid evolutionary rates in this region, in turn a consequence of short generation times, increased mutation rates at higher temperatures, and an acceleration of selection resulting from the former processes and the general increase of physiological processes at higher temperatures. This naturally begs the question of the extent to which generation time and number of generations vary over large geographic gradients, and the nature of any systematic patterns in this variation. Given little geographic variation in development rate, and the confounding effects thereon of body size and diet, as well as geographic variation in insect body size that regularly shows no consistent trends between groups, and is often the consequence of latitudinal changes in the dominance of higher taxonomic groups (Hawkins, 1995; Hawkins & Lawton, 1995), the actual form of the relationship between latitude and the number of generations seems remarkably difficult to determine. Broad overviews of insect voltinism do little more to clarify the situation. Thus, Wolda (1988) pointed out that although multivoltinism in tropical species is probably common, some species behave as if the environment is highly seasonal, thus resulting in broad variety of strategies in the tropics. Arguably the most that can be concluded from these works is that they are broadly suggestive of the possibility of a greater number of generations in the tropics than in more temperate regions. This leaves open the question of the contribution of voltinism to rapid evolutionary rates in the tropics.

In conclusion, it is clear that investigations of development rate are of more importance than just providing predictive models for insect pest management, apparently one of the major reasons for their execution (see Campbell & Mackauer, 1975; Nteletsana *et al.*, in press). Rather, they are also essential for informing a series of important questions in both physiological ecology and evolutionary biology.

References

Berrigan, D. & Partridge, L. 1997. Influence of temperature and activity on the metabolic rate of adult *Drosophila melanogaster*. *Comparative Biochemistry and Physiology* **118A**, 1301-1307.

Block, W. & Young, S.R. 1978. Metabolic adaptations of Antarctic terrestrial microarthropods. *Comparative Biochemistry and Physiology* **61A**, 363-368.

Bryant, S.R., Bale, J.S., Thomas, C.D. 1998. Modification of the triangle method of degree-day accumulation to allow for behavioural thermoregulation in insects. *Journal of Applied Ecology* **35**, 921-927.

Bullock, T.H. 1955. Compensation for temperature in the metabolism and activity of poikilotherms. *Biological Review* **30**, 311-342.

Campbell, A., Frazer, B.D., Gilbert, N., Gutierrez, A.P. & Mackauer, M. 1974. Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology* **11**, 431-438.

Campbell, A. & Mackauer, M. 1975. Thermal constants for development of the pea aphid (Homoptera: Aphididae) and some of its parasites. *Canadian Entomologist* **107**, 419-423.

Chown, S.L. 1997. Thermal sensitivity of oxygen uptake of Diptera from sub-Antarctic South Georgia and Marion Islands. *Polar Biology* **17**, 81-86.

Chown, S.L. & Gaston, K.J. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Review* **74**, 87-120.

Clarke, A. 1991. What is cold adaptation and how should we measure it? *American Zoologist* 31, 81-92.

Clarke, A. 1993. Seasonal acclimation and latitudinal compensation in metabolism: do they exist? *Functional Ecology* 7, 139-149.

Conover, D.O. & Present, T.M.C. 1990. Countergradient variation in growth rate: compensation for length of the growing season among Atlantic silversides from different latitudes. *Oecologia* **83**, 316-324.

Cossins, A.R. & Bowler, K. 1987. *Temperature Biology of Animals*. Chapman & Hall, London.

Gotthard, K., Nylin, S. & Wiklund, C. 1994. Adaptive variation in growth rate: life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. *Oecologia* **99**, 281-289.

Gotthard, K., Nylin, S. & Wiklund, C. 2000. Individual state controls temperature dependence in a butterfly (*Lassiommata maera*). *Proceedings of the Royal Society of London B* **267**, 589-593.

Hawkins, B.A. 1995. Latitudinal body-size gradients for the bees of the eastern United States. *Ecological Entomology* **20**, 195-198.

Hawkins, B.A. & Lawton, J.H. 1995. Latitudinal gradients in butterfly body sizes: is there a general pattern? *Oecologia* 102, 31-36.

Honêk, A. 1996a. Geographical variation in thermal requirements for insect development. *European Journal of Entomology* **93**, 303-312.

Honêk, A. 1996b. The relationship between thermal requirements for insect development: a verification. *Acta Societatis Zoologicae Bohomoslovenicae* **60**, 115-152.

Honêk, A. 1999. Constraints on thermal requirements for insect development. *Entomological Science* **2**, 615-621.

Honêk, A. & Kocourek, F. 1990. Temperature and development time in insects: A general relationship between thermal constants. *Zoologische Jahrbuecher Systematik* **117**, 401-439.

Lamb, R.J. 1992. Development rate of *Acyrthosiphum pisum* (Homoptera: Aphididae) at low temperatures: implications for estimating rate parameters for insects. *Environmental Entomology*, **21**, 10-19.

Levins, R. 1969. Thermal acclimation and heat resistance in *Drosophila* species. *American Naturalist* **103**, 483-499.

Levinton, J.S. 1983. The latitudinal compensation hypothesis: growth data and a model of latitudinal growth differentiation based upon energy budgets. I. Interspecific comparison of *Ophryotrocha* (Polychaeta: Dorvilleidae). *Biological Bulletin* **165**, 686-698.

McCullagh, P. & Nelder, J.A. 1989. *Generalized Linear Models*. Chapman and Hall, London.

Nteletsana, L., Schoeman, A.S. & McGeoch, M.A. (2001). Temperature effects on development and survival of the potato weevil, *Cylas puncticollis* Bohemia (Coleoptera: Apionidae). *African Entomology* (in press).

Rohde, K. 1992. Latitudinal gradients in species diversity: the search for the primary cause. *Oikos* **65**, 514-527.

Rohde, K. 1997. The larger area of the tropics does not explain latitudinal gradients in species diversity. *Oikos* **79**, 169-172.

Scholander, P.F.W., Flagg, V., Walters, W. & Irving, L. 1953. Climatic adaptation in arctic and tropical poikilotherms. *Physiological Zoology* **26**, 67-92.

Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*. Freeman & Co. New York.

Sømme, L. & Block, W. 1991. Adaptations to alpine and polar environments. In: Lee, R.E. and Delinger, D.L. (Eds.) *Insects at Low Temperature*. Chapman and Hall, London, pp. 318-359.

Trudgill, D.L. 1995. Why do tropical poikilothermic organisms tend to have a higher threshold temperatures for development than temperate ones. *Functional Ecology* **9**, 136-137.

Trudgill, D.L. & Perry, 1994. Thermal time and ecological strategies- a unifying hypothesis. *Annals of Applied Biology* **125**, 521-532.

Werner, E.E. & Anholt, B.R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *American Naturalist* **142**, 242-272.

Wolda, H. 1988. Insect seasonality: Why? *Annual Review of Ecology and Systematics*. **19**, 1-18.

Chapter 5

Metabolic cold adaptation and its hemispheric asymmetry in insects

Submitted to *Functional Ecology* for publication (2001)

5.1 Introduction

Ectotherms do not use metabolically produced heat to regulate body temperature, but several studies have suggested that, at the same trial temperature, species from cold climates have elevated metabolism compared to those from warm climates. This effect is referred to as metabolic cold adaptation (MCA), and it remains controversial (Clarke, 1993).

Numerous studies have argued in favour of MCA in many organisms (Rao & Bullock, 1954; Bullock, 1955; Sømme & Block, 1991; Ayres & Scriber, 1994). Cold water fish (and their isolated tissues) are known to exhibit an elevated metabolism, compared with warm-water fish, when tested at the same temperature (e.g. Wells, 1987; Torres & Somero, 1988). However, other studies have suggested that the concept cannot be upheld (Scholander *et al.*, 1953; Holeton, 1974; Steffensen *et al.*, 1994; Clarke & Johnston, 1999). Studies on other organisms other than fish, such as echinoderms, crustaceans, annelids and lamellibranch molluscs have also provided support for the presence of MCA (Fox, 1936, 1938, 1939; Fox & Wingfield, 1937; Hopkins, 1946).

Similarly, a number of studies on terrestrial species seem to support MCA in various invertebrates, including beetles (e.g. Aunaas *et al.*, 1983; Strømme *et al.*, 1986; Schultz *et al.*, 1992), grasshoppers (Chappell, 1983; Massion, 1983) and polar microarthropods (Block, 1977; Block & Young, 1978; Young, 1979). However, recent studies on midge (Lee & Baust, 1982a), a tick (Lee & Baust, 1982b), carabids (Nylund, 1991) and some fly species (Chown, 1997) found no evidence to support MCA. These are in support of early results by Scholander *et al.* (1953). In insects, MCA remains much disputed (Chown & Gaston, 1999).

It has also been argued that MCA should not only be included among the causes of interspecific variation in metabolic rate, which are of considerable importance in evolutionary ecology (McNab, 1980; Ricklefs *et al.*, 1996; Gorman *et al.*, 1998), but must also be added to the growing number of physiological variables in insects that show hemispheric asymmetry (Addo-Bediako *et al.*, 2000). This study is therefore to provide further evidence of variation in metabolic rate (MCA) in insects at a global scale, and the extent to which these patterns show phylogenetic components.

5.2 Materials and methods

To investigate MCA at global scales, published literature on insect standard metabolic rates as far back as 1900 were examined, but with the major emphasis on the last 50 years. From each study the following were extracted, data on metabolic rate, body mass, trial temperature(s) (including all data if more than a single trial temperature was used), latitude, longitude and altitude of the collection site (these variables often secondarily determined from gazeteers), experimental method (closed or flow through system respirometry), and wing status (flying or flightless, again often determined secondarily from the taxonomic literature). Data from studies where all of the above information could not be conclusively extracted, from immatures, from insects long maintained in laboratory colonies, and from studies where gas concentrations were manipulated, were excluded. Because metabolic rates are reported in several different ways, all values were converted to microwatts, assuming a respiratory quotient of 0.84 for studies reporting CO_2 production. Using an iterative procedure (Addo-Bediako *et al.*, 2000) to maximize latitudinal coverage of the database only single populations of each species where more than one population had been investigated was selected. This analysis included data on 347 species from 64 families and 12 orders and spanned the latitudinal range 54°S to 77°N. For each data point environmental temperature was determined by assigning a value, based on geographic position, from the half-degree mean annual temperature database (1961-1990) provided by the Intergovernmental Panel on Climate Change Data Distribution Centre (http://ipcc-ddc.cru.uea.ac.uk/ipcc_ddc/index.html). For investigations of collinearity of the independent variables, only single data points were used for each species.

5.3 Results and discussion

A generalized linear model (McCullagh & Nelder, 1989) was used to examine the effects of the continuous variables body mass, trial temperature, and environmental temperature, and the categorical variables experimental method (flow-through vs. closed system respirometry) and wing status (flying vs. flightless species), and their interaction term, on $\log_{10}$ metabolic rate. The model indicated, not unexpectedly, that body mass and trial temperature have a significant influence on metabolic rate (Peters, 1983; Cossins & Bowler, 1987). Experimental method, wing status, and environmental temperature also had a significant effect on metabolic rate, but the interaction term between experimental method and wing status were not significant (Table 5.1). The model took into account the significant, though weak, collinearity in the continuous, independent variables, such that metabolic rate in the largest insects was measured at the highest temperatures (trial temperature vs. body mass, $r_s = 0.113$, $p < 0.005$ with table-wide Bonferroni correction), larger insects were investigated in tropical areas (body mass vs. mean annual temperature, $r_s = 0.189$, $p < 0.05$), and thus metabolic rate in tropical insects was generally investigated at higher trial temperatures than metabolic rate in more cold-climate species (trial temperature vs. mean annual temperature, $r_s = 0.224$, $p < 0.01$).

Table 5.1. Generalized linear model of the explanatory variables on $\log_{10}$metabolic rate

Variables Goodness of fit	Parameter estimate	df (df)	Type III log-likelihood (Deviance)	Chi-square (Deviance/df)	p
Log_{10} mass	0.755	1	-813.32	1029	0.00001
Treatment temperature	0.037	1	-440.95	284.29	0.00001
Environmental temperature	-0.011	1	-323.58	49.55	0.00001
Wing status		1	-327.78	57.94	0.00001
Method		1	-333.08	68.54	0.00001
Wing status x method		1	-298.87	0.13	0.717
		(673)	(95.8)	(0.142)	

Although these results suggest that there is a weak, but significant negative relationship between mean annual temperature and metabolic rate, the significant effects of experimental method (in the direction suggested by Lighton & Fielden (1995), after accounting for all other effects, closed system mean = 262.7 μW; open system mean = 104.5 μW), and wing status (in the direction suggested by Reinhold

(Reinhold, 1999), after accounting for all other effects, winged species mean = 250.5 μW; flightless species mean = 109.5 μW) suggested that each combination of these two variables should be examined separately. For all four models, body mass and trial temperature had the expected positive effect on metabolic rate, and environmental temperature had a significantly negative effect (Table 5.2). Thus, at the interspecific level, metabolic cold adaptation, or temperature compensation, is clearly present in insects.

Table 5.2. Generalized linear model of the explanatory variables on $\log_{10}$metabolic rate in each of the four wing status by respirometry method groups

Explanatory variables (Goodness of fit)	Parameter estimate	df (df)	Type III log-likelihood (Deviance)	Chi-square (Deviance/df)	p
Flying species, closed system					
Log_{10} mass	0.704	1	-362.14	572.01	0.00001
Treatment temperature	0.043	1	-186.62	220.97	0.00001
Environmental temperature	-0.004	1	-78.66	5.04	0.025
		(329)	(30.80)	(0.094)	
Flying species, open system					
Log_{10} mass	0.500	1	-5.03	10.45	0.0004
Treatment temperature	0.040	1	-12.07	24.54	0.00001
Environmental temperature	-0.018	1	-3.29	6.98	0.005
		(28)	(1.85)	(0.066)	

Flightless species, closed system					
Log$_{10}$ mass	0.785	1	-304.41	298.31	0.00001
Treatment temperature	0.039	1	-186.66	62.78	0.00001
Environmental temperature	-0.015	1	-167.27	24.02	0.00001
		(239)	(51.06)	(0.214)	
Flightless species, open system					
Log$_{10}$ mass	0.758	1	-87.13	134.27	0.00001
Treatment temperature	0.042	1	-37.93	35.87	0.00001
Environmental temperature	-0.023	1	-24.73	9.46	0.00001
		(68)	(7.35)	(0.108)	

It has also been argued that the benefits of an elevated metabolic rate might accrue to cold-climate ectotherm species by way of a reduced sensitivity (i.e. lower slope) of the metabolic rate-temperature (R-T) curve (Rao & Bullock, 1954; Young, 1979). This second form of MCA is again polemical, with several authors supporting the idea (Bullock, 1955; Sømme & Block, 1991), and others opposing it (Scholander *et al.*, 1953; MacKay, 1982). To test this hypothesis, slopes of least-squares linear regressions of log rate on temperature were determined for each species individually where three or more trial temperatures had been used. In the case of multiple populations the above iterative protocol was used to select one population. Here, there was a significant negative relationship between environmental temperature and the slope of the R-T curves (Table 5.3), thus not providing support the reduced R-T sensitivity idea. However, some authors have also suggested that the environment might influence the shape of the R-T curve in more subtle ways (Tsuji 1988; Spicer & Gaston, 1999). For insects, it has recently been proposed (Chown, 1997; Chown & Gaston, 1999) that reduced sensitivity of metabolic rate to temperature might be characteristic of southern, cold-climate species, where climates are likely to be permanently cool and often cloudy (Danks, 1999). Under these conditions, opportunities for effective behavioural thermoregulation are limited (Chown, 1997). In northern, cold-climate species, where hot, sunny periods may be more frequent, thus facilitating behavioural thermoregulation (Danks, 1999; Gotthard *et al.*, 2000), greater R-T sensitivity would be expected. Hence, the metabolic benefits of R-T sensitivity that would accrue to species experiencing regularly sunny conditions in the northern hemisphere (Gotthard *et al.*, 2000; Nielsen *et al.*, 1999) should be outweighed by the costs associated with a permanently depressed metabolism under cloudy conditions in the southern hemisphere (Chown, 1997; Chown & Gaston, 1999). The data provide support for this idea. While there was no relationship between environmental temperature and slope of the R-T curve for the southern hemisphere, this was the case in the northern hemisphere (Table 5.3). This effect did not extend to metabolic rate variation as such, because elevated metabolic rates in cold climate species were found in both the Northern ($p < 0.00001$) and Southern ($P < 0.001$) hemispheres.

Table 5.3. Generalized linear model of the explanatory variables on the slope of the $\log_{10}$metabolic rate - temperature curve for all species, those found in the Northern Hemisphere only, and those found in the Southern Hemisphere only

Explanatory variables (Goodness of fit)	Parameter estimate	df (df)	Type III log-likelihood (Deviance)	Chi-square (Deviance/df)	p
All species					
Mass		1	321.62	1.04	0.31
Environmental temperature	-0.00028	1	319.69	4.90	0.03
Wing status		1	321.08	2.11	0.15
Method		1	321.98	0.30	0.58
Wing status x method		1	320.00	4.27	0.04
		(93)	(0.0087)	(0.000093)	
Northern Hemisphere					
Mass	0.0098	1	256.32	4.42	0.04
Environmental temperature	-0.00029	1	256.27	4.51	0.03
Wing status	0.00641	1	255.93	5.19	0.02
Method		1	257.36	2.27	0.13
Wing status x method		1	256.04	4.99	0.02
		(73)	(0.0067)	(0.000091)	

Southern Hemisphere

Mass	1	67.44	0.74	0.37
Environmental temperature	1	67.79	0.06	0.82
Wing status	1	67.79	0.04	0.84
Method	1	67.71	0.21	0.65
Wing status x method	1	67.08	1.47	0.22
	(14)	(0.0013)	(0.000095)	

Support for a genetic basis for these results is provided by a nested analysis of variance of metabolic rates, using single data points for each species, with all values corrected to 25°C assuming a Q_{10} of 2 (Cossins & Bowler, 1987; Reinhold, 1999). This indicated that 20% of the variance was accounted for at the species level, while 28% ($p < 0.001$) was partitioned at the level of genus, 31% ($p < 0.0001$) at family, and 21% ($p < 0.0001$) at order. This result also suggests that phylogenetic non-independence might have artificially elevated the degrees of freedom in this analysis (Harvey & Pagel, 1991). The absence of a phylogeny for the hexapods at the genus and family level, the small number of nodes available for analysis from a generic classification for the insects, and the fact that only 21% of the variation in metabolic rate is found at the order level, precluded an interpretable analysis of phylogenetically independent contrasts. Nonetheless, the same results were found when interspecific analyses of metabolic rate variation were repeated for Lepidoptera (all flying, $p < 0.05$) and Isoptera (all flightless, $p < 0.001$). Based on these additional analyses, and recent simulations (Ricklefs & Starck, 1996) and discussion (Bjorklund, 1997; Price, 1997), the conclusions are not unwarranted.

In sum, the results provide significant support for metabolic cold adaptation in insects, and highlight a further large-scale, hemispheric asymmetry in the physiology of insects. Such asymmetry may well contribute to large-scale differences in the biodiversity of the two hemispheres (Addo-Bediako *et al.*, 2000).

References

Addo-Bediako, A., Chown, S.L. & Gaston, K.J. 2000. Thermal tolerance, climatic variability and latitude. *Procedings of the Royal Society of London B* **267**, 739-745.

Aunaas, T., Baust, J.G. & Zachariassen, K.E. 1983. Ecophysiological studies on arthropods from Spitsbergen. *Polar Research* **1**, 235-240.

Ayres, M.P.& Scriber, J.M. 1994. Local adaptations to regional climates in *Papilio canadensis* (Lepidoptera: Papilionidae). *Ecological Monographs* **64**, 465-482.

Bjorklund, M. 1997. Are 'comparative methods' always necessary? Oikos **80**, 607-612.

Block, W. 1977. Oxygen consumption of the terrestrial mite *Alaskozetes antarcticus* (Acari: Cryptostigmata). *Journal of Experimental Biology* **68**, 69-87.

Block, W. & Young, S.R. 1978. Metabolic adaptatiosn of Antarctic terrestrial micro-arthropods. *Comparative Biochemistry and Physiology* **61A**, 363-368.

Bullock, T.H. 1955. Compensation for temperature in the metabolism and activity of poikilotherms. *Biological Reviews* **30**, 311-342.

Chappell, M.A. 1983. Metabolism and thermoregulation in desert and montane grasshoppers. *Oecologia* **56**, 126-131.

Chown, S.L. 1997. Thermal sensitivity of oxygen uptake of Diptera from sub-Antarctic South Georgia and Marion Islands. *Polar Biology* **17**, 81-86.

Chown, S.L. & Gaston, K.J. 1999. Why Rapoport's rule does not generalise. *Oikos* **84**, 309-312.

Clarke, A. 1993. Seasonal acclimatization and latitudinal compensation in metabolism: do they exist? *Functional Ecology* 7, 139-149.

Clarke, A. & Johnston, N.M. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology* **68**, 893-905.

Cossins, A.R. & Bowler, K. 1987. *Temperature Biology of Animals*. Chapman & Hall, London.

Danks, H.V. 1999. Life cycles in polar arthropods-flexible or programmed? *European Journal of Entomology* **96**, 83-102.

Fox, H.M. 1936. The activity and metabolism of poikilothermic animals in different latitudes. *Proceedings of the Zoological Society of London* **106A**, 945-955.

Fox, H.M. 1938. The activity and metabolism of poikilothermic animals in different latitudes. *Proceedings of the Zoological Society of London* **108A**, 501-505.

Fox, H.M. 1939. The activity and metabolism of poikilothermic animals in different latitudes. *Proceedings of the Zoological Society of London* **109A**, 141-156.

Fox, H.M. & Wingfield, C.A.1937. The activity and metabolism of poikilothermic animals in different latitudes. *Proceedings of the Zoological Society of London* **107A**, 275-282.

Harvey, P.H. & Pagel, M.D. 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.

Holeton, G.F. 1974. Metabolic cold adaptation of polar fish: fact or artifact? *Physiological Zoology* **47**, 137-152.

Hopkins, H.S. 1946. The influence of season, concentration of sea water and environmental temperature upon the oxygen consumption of tissues in Venus mercenaria. *Journal of Experimental Biology* **102**, 143-158.

Huey, R.B. & Berrigan, D. 1996. Phenotypic and evolutionary adaptation. In: Johnston, I.A. & Bennet, A.F. (Eds.) *Animals and Temperature*. Cambridge University Press, Cambridge.

Johnston, I.A., Clarke, A. & Ward, P. 1991. Temperature and metabolic rate in sedentary fish from Antarctic, North sea and Indo-West Pacific Ocean. *Marine Biology* **109** 191-195.

Lee, R.E. & Baust, J.G. 1982a. Absence of metabolic cold adaptation and compensatory acclimation in the Antarctic fly, *Belgica antarctica*. *Journal of Insect Physiology* **28**, 725-729.

Lee, R.E. & Baust, J.G. 1982b. Respiratory metabolism of the Antarctic tick, *Ixodes uriae*. *Comparative Biochemistry & Physiology* **72A**, 167-171.

Lighton, J.R.B. & Fielden, L.J. 1995. Mass scaling of standard metabolism in ticks: A valid case of low metabolic rates in sit-and-wait strategies. *Physiological Zoology* **68**, 43-62.

Mackay, W.P. 1982. An altitudinal comparison of oxygen consumption rates in three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). *Physiological Zoology* **55**, 367-377.

Massion, D.D. 1983. An altitudinal comparison of water and metabolic relations in two acridid grasshoppers (Orthoptera). *Comparative Biochemistry & Physiology* **74A**, 101-105.

McCullagh, P. & Nelder, J.A. 1989. *Generalised Linear Models*. Chapman and Hall, London.

McNab, B.K. 1980. Food habitats, energetics, and the population biology of mammals. *American Naturalist* **116**, 106-124.

Nielsen, M.G., Elmes, G.W. & Kipyatkov, V.E. 1999. Respiratory Q_{10} varies between populations of two species of *Myrmica* ants according to the latitude of their sites. *Journal of Insect Physiology* **45**, 559-564.

Nylund, L. 1991. Metabolic rates of *Calathus melanocephalus* (L.) (Coleoptera: Carabidae from Alpine and lowland habitats (Jeloy and Finse, Norway and Drenthe, The Netherlands). *Comparative Biochemistry & Physiology* **100A,** 853-862.

Peters, R.H. 1983. *The Ecological Implications of Body Size.* Cambridge University Press, Cambridge.

Price, T. 1997. Correlated evolution and independent contrasts. *Philosophical Transactions of the Royal Society, London* B **352**, 519-529.

Rao, K.P. & Bullock, T.H. 1954. Q_{10} as a function of size and habitat temperature in poikilotherms. *American Naturalist* **88**, 33-44.

Reinhold, K. 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology* **13**, 217-224.

Ricklefs, R.E., Konarzewski, M. & Daan, S. 1996. The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. *American Naturalist* **147**, 1047-1071.

Ricklefs, R.E. & Starck, J.M. 1996. Applications of phylogenetically independent contrasts: a mixed progress report. *Oikos* 77, 167-172.

Scholander, P.F., Flagg, W., Walters, V. & Irving, L. 1953. Climatic adaptation in Arctic and Tropical poikilotherms. *Physiological Zoology* **26**, 67-92.

Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*. Freeman & Co. New York.

Sømme, L. & Block, W. 1991. Adaptations to alpine and polar environments. In: Lee, R.E. & Delinger, D.L. (Eds.) *Insects at Low Temperature* pp. 318-359. Chapman and Hall, London.

Spicer, J.I. & Gaston, K.J. 1999. *Physiological Diversity and its Ecological Implications*. Blackwell Science.

Steffensen, J.F., Bushnell, P.G. & Schurmann, H. 1994. Oxygen consumption in four species of teleost from Greenland: No evidence for metabolic cold adaptation. *Polar Biology* **14**, 49-54.

Stromme, J.A., Ngari, T.W. & Zachariassen, K.E. 1986. Physiological adaptations in Coleoptera in Spitsbergen. *Polar Research* **4**, 199-204.

Todd, C.M. 1997. Respiratory metabolism in two species of carabid beetle from the sub-Antarctic Island of South Georgia. *Polar Biology* **18**, 166-171.

Torres, J.J. & Somero, G.N. 1988. Metabolism, enzymatic activities and cold adaptation in Antarctic mesopelagic fishes. *Marine Biology* **98**, 169-180.

Tsuji, J.S. 1988. Thermal acclimation of metabolism in *Sceloporus* lizards from different latitudes. *Physiological Zoology* **61**, 241-253.

Wells, R.G.M. 1987. Respiration of Antarctic fish from McMurdo Sound. *Comparative Biochemistry & Physiology* **88A**, 417-424.

Young, S.R. 1979. Respiratory metabolism of *Alaskozetes antarcticus*. *Journal of Insect Physiology* **25**, 361-369.

Chapter 6

Conclusion

In this study, a total of 2008 papers were examined, of which 437 (dated between 1926 and 1999) were concerned with lower lethal temperature (cold hardiness), 186 (1927-1999) with upper lethal temperature, 314 (1914-2000) with metabolic rate, 314 (1928-2000) with desiccation resistance (water balance), 615 (1932-2000) with the temperature dependence of development and 142 (1939-2000) with thermoregulation. Of course, not all work proved to be suitable for use for a variety of reasons (some of which are discussed below), and these were subsequently excluded from the analysis. Thus, a total of 1948 species belonging to 224 family and 20 orders were eventually used for these analyses. Appendix A provides a summary of the numbers of Orders, Families, Genera and Species studied for each variable, and Appendix B the full literature list, including studies that were excluded.

Rather than re-iterate the conclusions from each of the major sections, the aim of this section is to raise four issues that are critical both to insect comparative ecophysiology and to macrophysiology, using a summary of the data from the reviews that have been undertaken, as well as data on the extent of work on insect body temperatures / thermoregulation. These issues are:

- the extent to which variance in insect traits is partitioned at higher taxonomic levels,
- the prevalence of data for individual insect species on the full suite of physiological traits considered here,
- the geographic extent of insect physiological studies, and

- the frequency with which body size is reported in physiological studies.

Taxonomic variation

The work undertaken here has demonstrated that a considerable proportion of the variation in several physiological traits is partitioned at higher taxonomic levels (see Table 6.1 for a summary). These findings are not unusual, at least as far as can be ascertained from studies of the levels at which life history traits are partitioned in vertebrates (Read & Harvey, 1989; Harvey & Pagel, 1991). However, they do confirm the necessity of including in comparative insect physiological ecology, and macrophysiology, studies that control for phylogenetic non-independence (for further discussion see Harvey, 1996; Ricklefs, & Stark, 1996; Price, 1997; Blackburn & Gaston, 1998; Freckleton, 2000; Garland & Ives, 2000). Knowing the extent to which variance is partitioned at higher taxonomic levels can also provide insight into the extent to which variation in traits has a firm genetic basis (Chown, 2001).

Table 6.1. Distribution of variance in supercooling point (SCP) of freezing tolerant and intolerant insects, lower lethal temperature (LLT) of freezing tolerant insects, upper lethal temperature (ULT), critical thermal maximum (CTmax), metabolic rate, and water loss rate in insects. Tabulated values are percentage of the total variance accounted for at each successive level. The species level includes the error term in the data. (* $p < 0.05$, ** $p < 0.01$).

Variable	Order	Family	Genus	Species
Freezing intolerant SCP	18.68*	32.47**	33.14**	15.71
Freezing tolerant SCP	13.18	40.85**	44.12**	1.85
Freezing tolerant LLT	20.26	46.87**	0.06	32.81
Upper lethal temperature	0.72	46.61**	29.89**	22.78
CTmax	3.8	12.62	57.72**	25.86
Metabolic rate	21**	31**	28**	20
Water loss rate	13.95	24.51**	29.56**	31.98

Of course, the outcome of studies of variance partitioning will also depend on the groups included and the replication within them (see Chown *et al.*, 1999). Based on the data collected here, it appears that a large majority of the most speciose insect orders have been studied, although there are still some obvious gaps at this level (see Appendix A).

These findings suggest that there is room for additional work on basic physiological traits in a wide variety of species (see also Lighton, 1998). In so doing, the taxonomic coverage of the available knowledge of insect physiological traits might also be considered from a rather different viewpoint. That is, for how many species have the full range of physiological traits been examined? Although this question might at first appear trivial, it certainly is not. For example, in the section on water balance it was shown that metabolic rate and water loss are not independent. Thus, determining whether there is environment-related variation in such interactions, at a variety of scales, will clearly require comparative work on several species at a variety of locations. At the moment, this is unlikely to achieve using the published literature (Table 6.2). Indeed, information on development rate, water loss, upper and lower lethal temperature limits, metabolic rate, and body temperature are available for just three species: *Leptinotarsa decemlineata* (Coleoptera, Coccinellidae), *Apis mellifera* (Hymenoptera, Apidae) and *Drosophila melanogaster* (Diptera, Drosophilidae). For fewer combinations of variables there are more data available, though these numbers do not reflect the number of studies that have actually sought to identify the nature of the interactions between the variables. Such studies are much less common (see Punzo & Huff, 1989; Williams *et al.*, 1997 for examples). Obviously there is much scope for additional work on interactions between physiological variables and their spatial variation.

Table 6.2. Numbers of species for which an increasing number of each of six physiological variables (metabolic rate, water loss rate, development, upper thermal tolerance, lower thermal tolerance, and body temperature) have been investigated.

Number of physiological traits	Number of species
1	1479
2	339
3	79
4	39
5	10
6	3

Geographic extent

The majority of the work on insect physiological ecology has been undertaken in the Holarctic (Fig. 6.1). This is undoubtedly due largely to the global distribution of insect physiological ecologists, the majority of which reside in this region. Nonetheless, together with the outcomes of the analyses these maps (Figs. 6.1 & 6.2) highlight several important, and in some instances concerning, trends.

Foremost among these trends is the tendency for investigations of particular traits to be biased to certain geographic regions. Thus, studies of lower lethal temperatures of insects tend to be undertaken in cold regions, and those of upper lethal temperatures in warm, often arid regions (Fig. 6.2). Likewise, data on development of tropical species are sparse, and it is also clear that investigations of desiccation resistance tend to be most common in desert regions. Thus, for the moment, tests of macroecological assumptions, such as the physiological tolerance assumption underlying the climatic variability explanation for Rapoport's rule, will have to rely

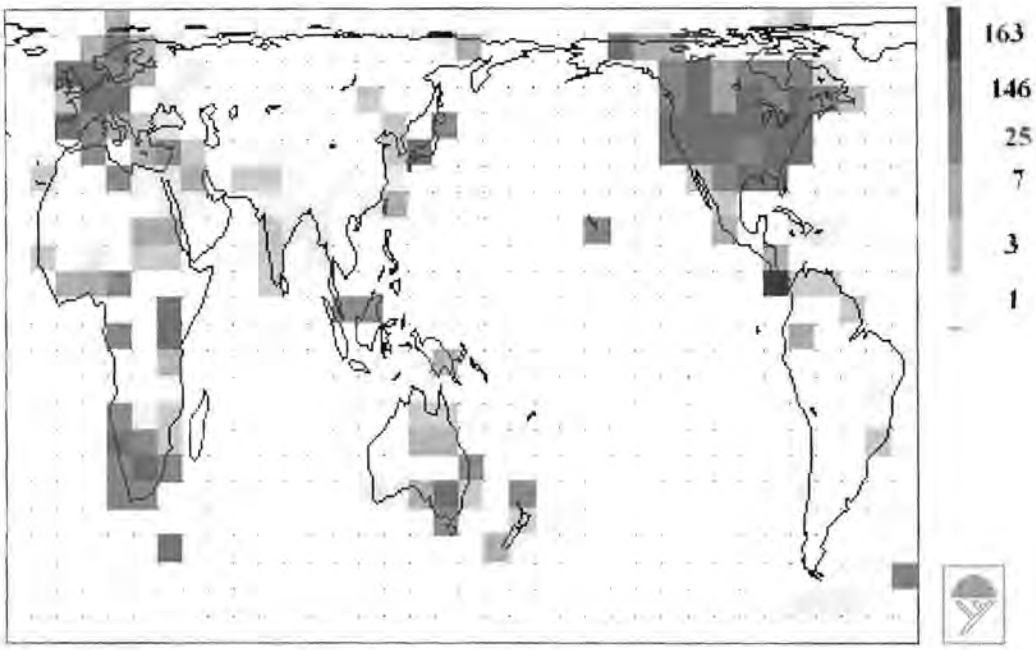

Fig. 6.1. Global geographic extent of investigations of metabolic rate, water loss, development rate, upper lethal temperature, lower lethal temperature, and body temperature in insects. This map was produced using WorldMap IV, based on a survey of a total of 2008 published works mainly from the English literature. The grey scale indicates the number of species examined.

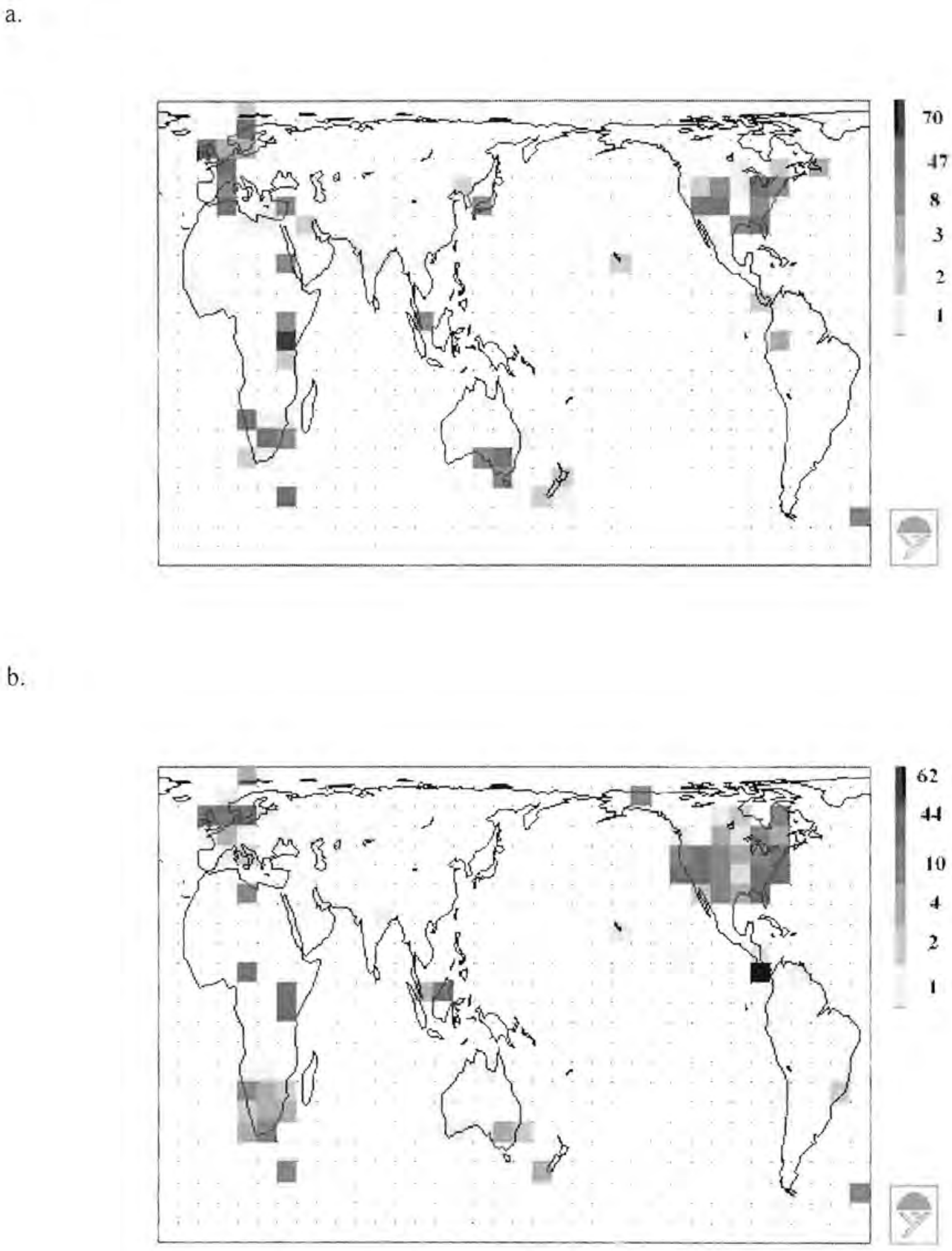

Fig. 6.2. Global geographic extent of investigations in insects of a). water loss, b). metabolic rate. The grey scale indicates the number of species examined.

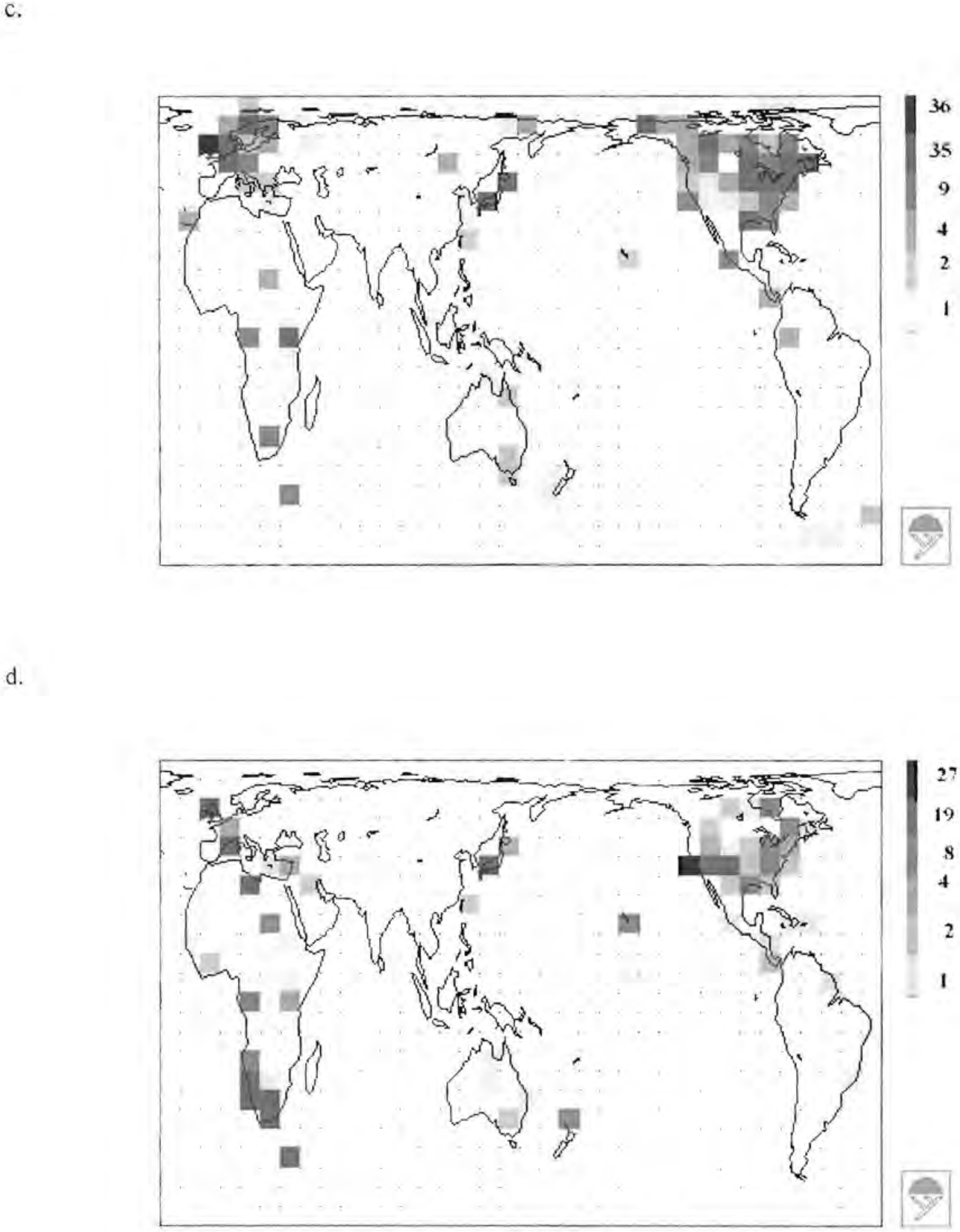

Fig. 6.2 Global geographic extent of investigations in insects of c). lower lethal temperature including supercooling point, d). upper lethal temperature. The grey scale indicates the number of species examined.

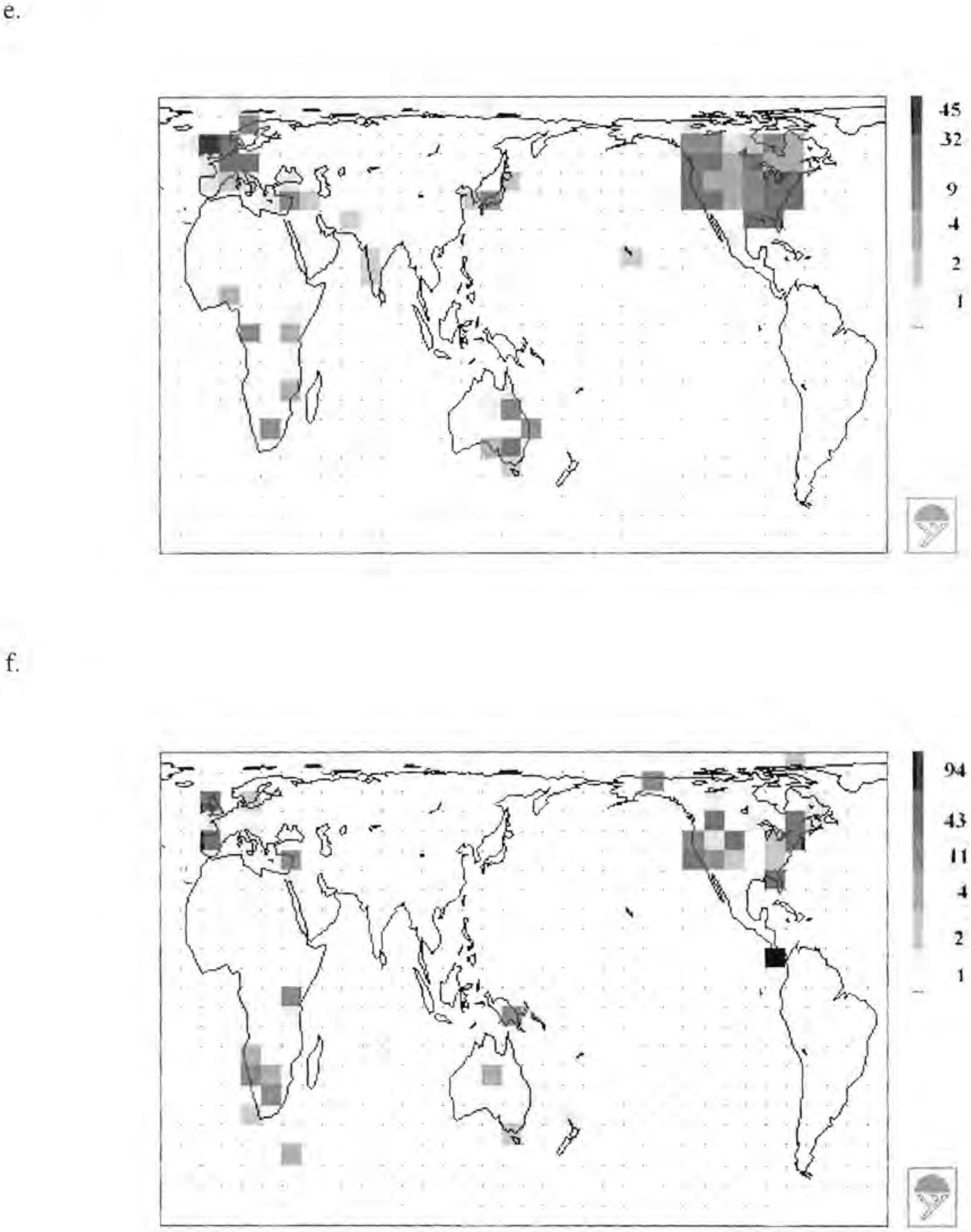

Fig. 6.2. Global geographic extent of investigations in insects of e). development time, f). body temperature. The grey scale indicates the number of species examined.

on information that is at best patchy (see for instance the resampling exercise undertaken for the thermal tolerance study because of data scarcity in the tropics).

Second, sound macrophysiological data might provide valuable insight into macroecological patterns that are scarcely documented, let alone clearly understood. For example, hemispheric asymmetry in species richness patterns and in patterns of range size variation have been documented in many groups (Gaston, 1996; Gaston and Blackburn, 2000). Species richness gradients tend to be less steep in the southern hemisphere, as do declines in range size. Similar, pronounced hemispheric asymmetry was found in lower lethal temperatures, with Southern Hemisphere species showing reduced cold hardiness, and a preponderance of freezing tolerance, compared with their Northern Hemisphere counterparts (see also Klok & Chown, 1997). This asymmetry might go some way towards explaining the absence of a Rapoport effect in many southern taxa (Gaston *et al*., 1998). A similar hemispheric asymmetry exists for the thermal sensitivity of metabolic rate, and suggests that regular differences between the two hemispheres deserve further exploration.

In sum, both macorecology and macrophysiology could benefit substantially from an increase in the geographic extent of work in insect physiological ecology. Indeed, it appears that rather than filling the "matrix" (see Lighton, 1998 for discussion), insect physiological ecologists might have some way to go before its form is clearly revealed.

Body size

It is widely appreciated that body size and many physiological variables are highly correlated, and that interactions between the latter and life history variables often produce the range in body sizes that have been documented for various assemblages (Peters, 1983; Schmidt-Nielsen, 1984; Chown & Gaston, 1997; Koslowski & Weiner, 1997). This raises an obvious question: how often is body size reported in the published literature?

Nee and Lawton (1996) rightly pointed out that that ecologists and physiologists apparently record body sizes as frequently as journalists report people's ages. The same appears to be true at least for some of the studies we have examined (Table 6.3). However, the regularity with which body size is reported depends critically on the variable that was of interest to the authors. Thus, unsurprisingly, body size is regularly reported in studies of insect water loss and metabolic rate. However, despite the fact that body size is thought to influence both cold hardiness (via water content) (see Block, 1990), and rate of development (Honêk, 1999), it is rarely reported in studies of these variables and upper thermal limits.

Table 6.3. The extent to which body size is reported, given as a percentage of the papers reviewed in that field (n), in investigations of five physiological variables.

Physiological trait	Percentage	n
Lower lethal temperature	15	268
Upper lethal temperature	8	141
Metabolic rate	76	170
Water loss rate	64	129
Development	6	180

Such neglect of a variable that is known either to influence physiological traits, or at least be closely correlated with them, is not unusual. For example, although wing status is known to have a pronounced effect both on water loss rate (Hesse, 1938; Cloudsley-Thompson, 1964; Draney, 1993; Davis *et al*., 2000) and metabolic rate (Reinhold, 1999), the wing status of insects is rarely reported. Nonetheless, it is a situation that should be remedied in all future studies. Body mass is one of the easiest variables to record, and substantially more so than many of the physiological variables that are routinely studied. Simply providing this measure in future physiological studies will place both comparative physiological and macrophysiological analyses on a much firmer footing.

Thus, and in conclusion, a resurgence of interest in large-scale phenomena, and the desire for information on the physiological assumptions underlying explanations for these patterns has meant that there is much that physiological ecology can to contribute to ecology and evolutionary biology in general. Indeed, there is scope for fruitful interactions across all levels in the physiological and ecological hierarchies, from work such as that on the cellular basis of thermal tolerance (e.g. Feder & Hoffmann, 1999), to broad scale investigations of physiological tolerances. However, for this to happen, there is still much that needs to be done that might strike many physiological ecologists as boring or "matrix filling". However, without at least some work that seeks to enlarge the geographic and taxonomic extent of the research done to date, physiological ecology might not enjoy as fruitful an interaction with macroecology and evolutionary biology as otherwise would be possible.

References

Blackburn, T.M. & Gaston, K.J. 1998. Some methodological issues in macroecology. *American Naturalist* **151**, 68-83.

Block, W. 1990. Cold tolerance of insects and other arthropods. *Philosophical Transactions of the Royal Society, London B* **326**, 613-633.

Chown, S.L. 2001. Physiological variation in insects: hierarchical levels and implications. *Journal of Insect Physiology* (in press).

Chown, S.L. & Gaston, K.J. 1997. The species-body size distribution: energy, fitness and optimality. *Functional Ecology* **11**, 365-375.

Chown, S.L., Le Lagadec, M.D. & Scholtz, C.H. 1999. Partitioning variance in a physiological trait: desiccation resistance in keratin beetles (Coleoptera: Trogidae). *Functional Ecology* **13**, 838-844.

Cloudsley-Thompson, J.L. 1964. On the function of the sub-elytral cavity in desert tenebrionidae (Col.). *Entomologist's Monthly Magazine* **100**, 148-151.

Davis, A.L.V., Chown, S.L., McGeoch, M.A. & Scholtz, C.H. 2000. A comparative analysis of metabolic rate in six Scarabaeus species (Coleoptera: Scarabaeidae) from the southern Africa: further caveats when inferring adaptation. *Journal of Insect Physiology* **46**, 553-562.

Draney, M.L. 1993. The subelytral cavity of desert tenebrionids. *Florida Entomologist* **76**, 539-548.

Feder, M.E. & Hoffmann, G.E. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annual Review of Physiology* **61**, 243-282.

Freckleton, R.P. 2000. Phylogenetic tests of ecological and evolutionary hypothesis: checking for phylogenetic independence. *Functional Ecology* **14**, 129-134.

Garland, T. & Ives, A.R. 2000. Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods. *American Naturalist* **155**, 346-476.

Gaston, K.J. 1996. Biodiversity-latitudinal gradients. *Progress in Physical Geography* **20**, 466-476.

Gaston, K.J. & Blackburn, T.M. 2000. *Pattern and Progress in Macroecology*. Blackwell Science, Oxford.

Gaston, K.J. , Blackburn, T.M. & Spicer, J.I. 1998. Rapoport's rule: time for an epitaph? *Trends in Ecology and Evolution* **13**, 70-74.

Harvey, P.H. 1996. Phylogenies for ecologists. *Journal of Animal Ecology* 65, 255-263.

Harvey, P.H. & Pagel, M.D. 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.

Hesse, A.J. 1938. some adaptive responses of insect life to semi-arid conditions in South Africa. *South African Journal of Science* **35**, 69-92.

Honěk, A. 1999. Constraints on thermal requirements for insect development. *Entomological Science* **2**, 615-621.

Klok, C.J. & Chown, S.L. 1997. Critical thermal limits, temperature tolerance and water balance of a sub-Antarctic caterpillar, *Pringleophaga marioni* Viette (Lepidoptera: Teneidae). *Journal of Insect Physiology* **43**, 685-694.

Koslowski, J. & Weiner, J. 1997. Interspecific allometrics are by-products of body size optimisation. *American Naturalist* **149**, 352-380.

Lighton, J.R.B. 1998. Notes from the underground: towards ultimate hypothesis of cyclic, discontinuous gas-exchange in tracheate arthropods. *American Zoologist* **38**, 483-491.

Nee, S. & Lawton, J.H. 1996. Ecology-body-size and biodiversity. *Nature* **380**, 672-673.

Peters, R.H. 1983. *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge.

Price, T. 1997. Correlated evolution and independent contrasts. *Philosophical Transactions of the Royal Society, London B* **352**, 519-529.

Punzo, F. & Huff, G. 1989. Comparative temperature and water relations and the effects of thermal acclimation on *Tenebrio molitor* and *Tenebrio obscurus* (Coleoptera: Tenebrionidae). *Comparative Biochemistry and Physiology* **93A**, 527-533.

Read, A.F. & Harvey, P.H. 1989. Life history differences among the eutherian radiations. *Journal of Zoology, London* **219**, 329-353.

Reinhold, K. 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Functional Ecology* **13**, 217-224.

Ricklefs, R.E. & Starck, J.M. 1996. Applications of phylogenetically independent contrasts: a mixed progress report. *Oikos* 77, 167-172.

Schmidt-Nielsen, K. 1984. *Scaling. Why is Animal Size so Important?* Cambridge University Press, Cambridge.

Williams, A.E., Rose, M.R. & Bradley, T.J. 1997. CO_2 release patterns in *Drosophila melanogaster*: The effect of selection for desiccation resistance. *Journal of Experimental Biology* **200**, 615-624.