

## Physiological climatic limits in *Drosophila*: patterns and implications

A. A. Hoffmann

The University of Melbourne, Bio21 Institute, 30 Flemington Road, Parkville, Victoria 3052, Australia  
ary@unimelb.edu.au

Accepted 29 October 2009

### Summary

Physiological limits determine susceptibility to environmental changes, and can be assessed at the individual, population or species/lineage levels. Here I discuss these levels in *Drosophila*, and consider implications for determining species susceptibility to climate change. Limits at the individual level in *Drosophila* depend on experimental technique and on the context in which traits are evaluated. At the population level, evidence from selection experiments particularly involving *Drosophila melanogaster* indicate high levels of heritable variation and evolvability for coping with thermal stresses and aridity. An exception is resistance to high temperatures, which reaches a plateau in selection experiments and has a low heritability/evolvability when temperatures are ramped up to a stressful level. In tropical *Drosophila* species, populations are limited in their ability to evolve increased desiccation and cold resistance. Population limits can arise from trait and gene interactions but results from different laboratory studies are inconsistent and likely to underestimate the strength of interactions under field conditions. Species and lineage comparisons suggest phylogenetic conservatism for resistance to thermal extremes and other stresses. Plastic responses set individual limits but appear to evolve slowly in *Drosophila*. There is more species-level variation in lower thermal limits and desiccation resistance compared with upper limits, which might reflect different selection pressures and/or low evolvability. When extremes are considered, tropical *Drosophila* species do not appear more threatened than temperate species by higher temperatures associated with global warming, contrary to recent conjectures. However, species from the humid tropics may be threatened if they cannot adapt genetically to drier conditions.

Key words: limits, climate change, evolution, *Drosophila*, DNA decay.

### Introduction

Physiological limits occur when the abiotic conditions of an organism's environment become stressful, reducing fitness to the extent that organisms fail to reproduce and survive, potentially leading to the extinction of populations and even species (Hoffmann and Parsons, 1991). Physiological limits can influence the susceptibility of organisms to climate change and indicate vulnerability (Bernardo et al., 2007; Huey et al., 2009; Williams et al., 2008). The vulnerability of terrestrial ectotherms is likely to be dictated by the proximity of the environment they experience to their physiological limits within the context of future climate change, and this may make groups like tropical insects and lizards particularly susceptible to extinction even when predicted thermal changes in the tropics are less than in other areas (Deutsch et al., 2008; Huey et al., 2009). Vertebrate populations from lowland areas may also be particularly vulnerable because they occur close to their thermal threshold and are already experiencing stressful conditions (Bernardo and Spotila, 2006; Huey et al., 2009). The distance between limits and conditions experienced by organisms provides a way of assessing vulnerability, although this is not a trivial task because organisms are exposed to multiple stressors whose importance may vary in time and space at different scales and whose impacts on distributions might only depend on rare events (Denny et al., 2006; Elton, 1930; Helmuth, 2009).

In ectotherms, physiological limits are often considered by plotting the performance of an organism on the *y*-axis against a variable (typically temperature) along the *x*-axis. The fertility and viability under different rearing temperatures for *Drosophila melanogaster* are plotted in this manner in Fig. 1. Such performance curves form the basis of theoretical and conceptual

approaches to understanding thermal adaptation (Angilletta, 2009; Gilchrist, 1995; Huey and Kingsolver, 1989). The position of an individual along the environmental gradient dictates its proximity to a physiological limit where performance declines to zero, and this differs depending on the trait considered (covering a narrower range of temperatures for fertility than viability in Fig. 1). In nature, organisms also often experience short periods of stressful conditions that extend outside the range of conditions in which organisms can be cultured (Fig. 1). The thermal extremes that can be tolerated in these short periods typically depend on phenotypic plasticity. At the individual level, physiological limits therefore depend on the trait being evaluated, length of exposure of the extreme conditions and plastic responses. Limits also depend on any behavioural responses that influence the microclimate experienced by individuals (Huey et al., 2003; Marais and Chown, 2008) (Table 1).

Evolution might alter performance curves (and the effect that plastic and behavioural responses have on these curves) over time. Physiological limits at the population and species levels encompass these evolutionary shifts (Table 1). Evolutionary biologists have often emphasised the large amounts of genetic variation that exist for traits within populations and the extent to which there are numerous genes and genetic options available for evolutionary shifts (Flint and Mackay, 2009), with mutation continually producing new variation that can persist in populations when traits are under stabilising selection (Zhang and Hill, 2005). Theoretical models (e.g. Lynch and Lande, 1993) provide a picture of how performance optima and limits might evolve under changing environmental conditions and the rate of environmental change that can be countered by organisms through evolution.

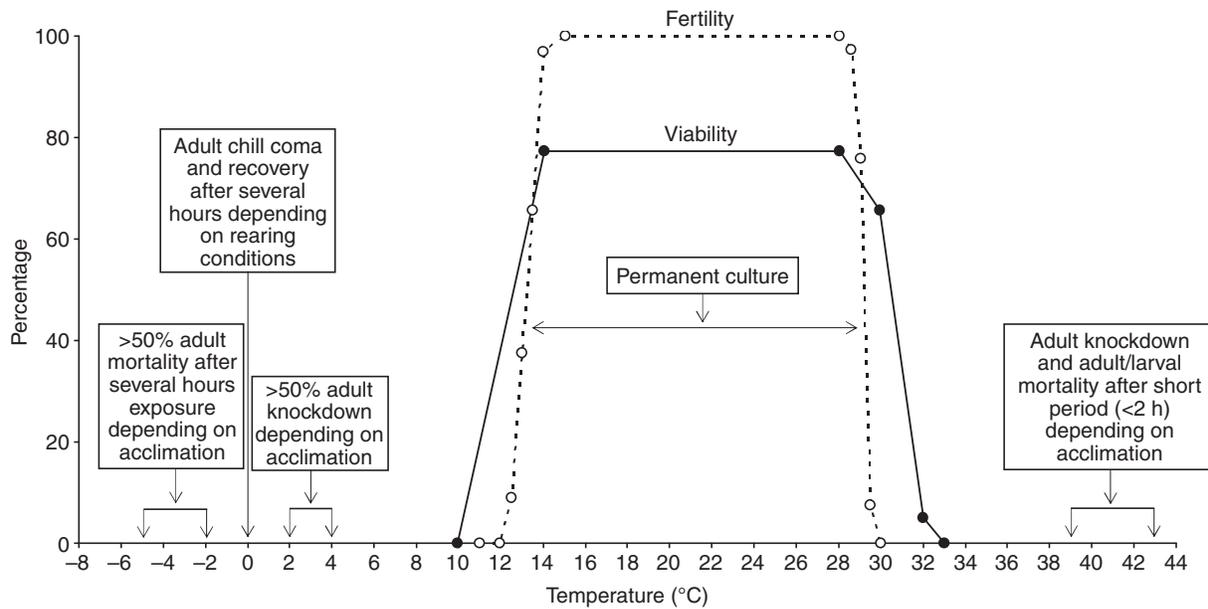


Fig. 1. Response of *Drosophila melanogaster* to thermal conditions for multiple traits. This species exhibits high viability across a wide range of culture temperatures (14–28°C). Adult fertility is maintained across a somewhat narrower range. Cold and hot extremes that produce incapacitation and death after a few hours extend well outside this range of temperatures. Based on a variety of sources (David et al., 2005; Hoffmann et al., 2003; Rako and Hoffmann, 2006).

Physiological limits at the population level also encompass demographic factors including stochasticity in population size. If a population that fluctuates in size is close to a physiological limit, it is more likely to go extinct than a population that is always maintained at a large size (Burger and Lynch, 1995; Lynch and Lande, 1993). There is also an interaction between population size and the evolutionary potential of populations, because genetic variation for evolutionary responses tends to be lost in small and fluctuating populations (Lynch and Lande, 1993; Willi et al., 2006).

Several explanations have been proposed to account for physiological limits at the population (and species) levels (Table 1). One of these is that a lack of genetic variation for traits dictates physiological limits. Another explanation is that genetic variation does exist but cannot be selected because of strong trait and genetic interactions. A third explanation is that limits might be modified

by evolution but evolution is prevented because gene flow is continually bringing poorly adapted genotypes into a population that is under selection.

A selection limit due to a lack of genetic variance was proposed by Bradshaw to account for the fact that only some plant species evolved resistance to soil contaminants (Bradshaw, 1991). This proposal has not been considered seriously by many evolutionary biologists due to the multitude of studies that have demonstrated genetic variation in quantitative traits. Hansen and Houle for instance suggest that morphological traits are almost always variable genetically even when traits show stasis over historical and paleontological time (Hansen and Houle, 2004). However, in some cases it does appear that levels of genetic variation for physiological traits can be low enough to act as a limit (Blows and Hoffmann, 2005).

Stasis in physiological limits can also occur when genetic variation is present in populations (Merila et al., 2001). In this

Table 1. A hierarchy of physiological limits

Level	Nature of limit	Modification of limit?	Signature of limit
Individual	Inability of individual to counter stressful conditions	Through diapause, hardening, acclimation, migration and behaviour	Reproductive failure, loss of mobility, loss of recovery
Population	Demographic: fluctuations in population size as limits are approached	Depends on the ability to recover and connectivity with other populations	Increased fluctuations in size, local extinction
	Evolutionary: inability to mount evolutionary response due to lack of appropriate genetic variation (due to directional selection, DNA decay or trait interactions)	Through altered trait interaction and selection patterns, altered connectivity between locally adapted populations, genetically based plastic responses	Reduction in population size and increased fluctuations in size, lack of fitness recovery as stressful conditions continue
	Evolutionary: inability to mount evolutionary response due to population dynamics in landscape	Altered patterns of gene flow that facilitate evolutionary responses	Loss of adaptive differentiation along environmental gradient, lack of fitness recovery
Species	Fundamental physiological/biochemical limit	Through gene rearrangement, hybridisation/introgression	Reduction in species distribution and abundance, extinction
Lineage	Phylogenetic limit	Genome reorganisation, evolution of new functions	Niche conservatism, phylogenetic signature in stress response

case, other factors might be involved, such as an uncoupling of the expression of genetic variance and selection (Wilson et al., 2006), or the presence of genetic variation for traits but not in the direction in which selection is acting (Blows, 2007). Stasis of traits may be associated with the integration of characters (pleiotropic constraints) and the integration of genes (epistatic constraints) (Hansen and Houle, 2004). Gene flow might play a role in limiting adaptation particularly at species margins where populations may be exposed to a directional influx of genes from large central populations adapted to different conditions (Holt and Gaines, 1992; Kirkpatrick and Barton, 1997) as reviewed in Bridle et al. (Bridle et al., 2009b).

If these constraints act to restrict evolutionary responses in populations, they may result in fundamental tolerance limits at the species level (Table 1). Limits might then only be broken through substantial genetic rearrangement, such as the acquisition of new resistance genes following hybridisation and introgression (Besansky et al., 2003). If these rearrangements are not possible, there may be phylogenetic limits and niche conservatism in related lineages of species. There is some indirect evidence of niche conservatism, such as the fact that invasive populations often reach high latitude limits similar to those of populations from where they originated rather than evolving to new limits (Wiens and Graham, 2005), although this pattern is not universal due to other factors determining distribution limits (Duncan et al., 2009).

Genetic variation in physiological traits has been studied more intensively in *Drosophila* than in other insects and a variety of approaches have been used (Table 2). Here I review different levels at which limits can be investigated including the individual, population, species and lineage levels. The importance of different factors determining limits at these levels is considered. Finally I consider ways in which *Drosophila* data might be used in predicting the susceptibility of species to climate change.

### Individual limits

As already mentioned, thermal limits in *Drosophila* species are dependent on the types of traits that are used to assess them as well as life-cycle stage. This has been well established for *D. melanogaster* through work by David and colleagues (David et al., 2004). In this species, egg-to-adult viability is limited to the range 10–32°C (Petavy et al., 2001) whereas male fertility has a narrower range from 12°C to 30°C (Chakir et al., 2002) (Fig. 1). Adult knockdown and mortality occurs well beyond these limits. For instance flies exposed to 4°C after being reared at 19°C show partial knockdown but then completely recover again (Rako and Hoffmann, 2006). However, this recovery is not evident when flies are reared at 25°C rather than 19°C, illustrating that thermal limits are dramatically influenced by the conditions flies experience. *Drosophila* larvae are usually more susceptible to thermal extremes than are adults (Hoffmann et al., 2003).

Thermal limits depend on the rate at which temperatures are altered and also on behaviour (Table 2). For adult knockdown under hot conditions, the upper thermal limit is lowered by around 1.5°C when temperature is increased slowly at a rate of 0.1°C per minute, compared with rapidly at a rate of 0.5°C per minute (Chown et al., 2009). However, repeated exposures to sublethal hot conditions increase resistance rather than lowering limits (Krebs and Loeschcke, 1994). In the field, adults can avoid extreme conditions and change the level of thermal stress they experience by seeking out microhabitats or through long-distance migration (Dillon et al., 2009; Feder et al., 2000; Kimura and Beppu, 1993) and this includes entering buildings under very cold conditions. These factors can make it difficult to evaluate the proximity of adult *Drosophila* to their thermal limits in nature (Table 2).

For aridity, individual limits depend on the nature of the trait scored. Flies selected for desiccation resistance can survive for 24 h or more of exposure to desiccant that reduces humidity to 10%

Table 2. Approaches for testing and recognising *Drosophila* tolerance limits in nature

Level	Approach	Done?	Examples
Individual	Rear individuals under a range of conditions relevant to nature	Ample information on constant temperature responses, less on variable conditions	(David et al., 2005)
	Expose individuals to relevant short-term environmental modifications	Rarely, exposures normally involve rapid transfer to stressful conditions	(Czajka and Lee, 1990)
	Compare diapause and non-diapause individuals	Some tests on limits following adult diapause completed	(Schmidt and Paaby, 2008)
	Incorporate behaviour into laboratory or field assessments	A few field releases where behaviour possible, also observations on activity time	(Huey and Pascual, 2009)
Population	Demographic studies	Rarely, limited to laboratory comparisons	(Willi and Hoffmann, 2009)
	Studies incorporating species interactions	Rarely, limited to laboratory comparisons	(Davis et al., 1998)
	Heritability studies	Several estimates of heritability of limits and genetic correlations but mostly on <i>D. melanogaster</i> , and focus is on ease of scoring traits rather than ecological realism	(Kellermann et al., 2009)
Inter-population	Selection experiments	Several experiments but as above for heritability studies	(Bubly and Loeschcke, 2005; Gilchrist and Huey, 1999)
	Natural population studies at margins or along clines Geographical variation	Some estimates of gene flow influencing limits Numerous studies of geographical variation in traits related to limits	(Bridle et al., 2009a; Jenkins and Hoffmann, 1999) (Hoffmann et al., 2002)
Species	Historical across past climate to test whether distribution tracks changes in climate	Has not been undertaken in a comprehensive manner	(Kimura et al., 1994)
	Testing invasive range of species when compared with native range	Rare, few examples	(Prevosti et al., 1988)
Lineage	Species comparisons	Several species comparisons suggest phylogenetic limits	(Kimura, 2004; Matzkin et al., 2009)

or less but adult mating success is affected after exposures of only a few hours (Gefen and Gibbs, 2009). Limits might be modified by plastic responses, because prior exposure to dry conditions can modify resistance levels at least in some *Drosophila* species (Hoffmann, 1990). As in the case of thermal limits, behaviour can modify the proximity of flies to aridity limits in nature (Prince and Parsons, 1977) but this is rarely considered (Table 2).

With multiple limits modified by environmental conditions, which limits are ecologically relevant, affecting the ability of species to survive and reproduce in a particular region? Under cold winter conditions, *D. melanogaster* and other species typically survive at the adult stage. Adult survival is enhanced by thermal conditions that promote reproductive diapause (Schmidt et al., 2005), which in turn increases cold resistance (Schmidt and Paaby, 2008). Larvae do not survive winter conditions in temperate areas (Mitrovski and Hoffmann, 2001). Limits affecting late reproduction and the maintenance of fertility are important in adults because overwintering flies have to reproduce in spring when conditions become favourable (Mitrovski and Hoffmann, 2001). In summer, both larvae and adults may suffer high levels of mortality from heat stress (Feder et al., 1997; Kristensen et al., 2007). Limits to heat stress are altered both by rapid hardening that extends thermal limits but also by diurnal fluctuations in temperature that decrease limits (Loeschcke and Hoffmann, 2007; Terblanche et al., 2007). Low levels of humidity in summer will further restrict adult activity and affect adult mating behaviour and survival (Gibbs et al., 1998).

Behavioural and plastic effects can make it hard to characterise individual limits of *Drosophila* species. Ideally different life-cycle stages should be considered and fluctuating environmental conditions reflecting those found in nature should be used. Researchers working on both larval and adult stages have considered the effects of natural fluctuations in temperature just prior to the development of stressful conditions (Kely and Lee, 1999) but there have been few attempts to assess the effects of naturally fluctuating conditions experienced by immature stages on resistance in later developmental stages.

Individual limits depend not only on plastic effects like acclimation and diapause but also on genotype. With ample evidence for genetic variation in responses to thermal extremes (Hoffmann et al., 2003) as well as desiccation (Gibbs et al., 2003), conditions that are stressful for one individual might not be stressful for another, raising the possibility of evolution at the population level shifting physiological limits.

#### Population limits: demographic factors

When population sizes are relatively small and fluctuate, there is a possibility of populations going extinct due to demographic and environmental stochasticity. Environmental stochasticity is likely to occur when individuals within populations are close to physiological limits and therefore have an increased risk of reproductive failure and death. The role of both types of stochasticity in driving local extinctions in nature has not been examined in *Drosophila* populations (Table 2). Perhaps demographic extinctions are unlikely in this group because populations of *Drosophila* species are typically very large and/or capable of expanding rapidly when resources suitable for breeding are available. Local extinctions of *Drosophila* populations due to demographic factors might also rarely be documented because of the inconspicuous nature of flies and lack of information about populations of rare species, many of which remain undescribed.

Stochastic processes have however been examined in experimental populations of *Drosophila*. For instance, in the rainforest species *D. birchii* exposed to heat stress, demographic factors tend to dictate the fate of populations when the size of the

adult population is held at 20, while both genetic and demographic factors are important in populations of size 100, and genetic factors are more important than demographic factors when a population size of 1000 individuals is maintained (Willi and Hoffmann, 2009). Population limits will also be influenced by interactions among species, both through competitive interactions and predation/parasitism, as shown in laboratory manipulations of multiple *Drosophila* species (Davis et al., 1998).

#### Population limits: genetic variation

Genetic variation can be determined through heritability studies and selection experiments (Table 2). Numerous studies on *D. melanogaster* have highlighted the presence of genetic variation for physiological traits [Burke and Rose, and others (Burke and Rose, 2009; Gibbs, 1999; Hoffmann et al., 2003) and references therein]. These include traits associated with thermal responses, starvation resistance and responses to aridity. The general conclusion from this body of work is that there is ample genetic variation to generate similarity between parents and offspring, and to produce responses to selection imposed artificially or as part of a program of experimental evolution. These conclusions have been backed up by more limited work on other species including *Drosophila buzzatii* (e.g. Sorensen et al., 1999) and *Drosophila simulans* [David et al. (David et al., 2004) and references therein].

However, it may not always be possible to extrapolate results from traits scored in laboratory conditions to those likely to be experienced in the field. This issue is illustrated by adult heat resistance. In *D. melanogaster* this trait shows a low heritability of around 10–20% when scored from selection experiments where flies are exposed immediately or with a fast ramping rate to high temperatures (Gilchrist and Huey, 1999; McColl et al., 1996). However, adults are often likely to experience slower ramping rates in nature of 0.06 to 0.1°C per minute. Under these conditions, the maximum temperature that *D. melanogaster* can tolerate decreases markedly (Chown et al., 2009), as also found in tsetse flies (Terblanche et al., 2007). There is a concomitant increase in phenotypic variance that might reflect an increase in environmental variance leading to a decrease in heritability (Chown et al., 2009). Ramping effects on heritability were recently shown directly by Mitchell and Hoffmann (Mitchell and Hoffmann, 2010); parent–offspring comparisons indicated that the heritability for adult knockdown under ramping in *D. melanogaster* was very close to zero despite small standard errors of 0.07 or less, compared with around 15–20% when flies were directly stressed without ramping. The low heritability in the ramping assay was associated with a loss of additive genetic variance as well as an increase in environmental variance, leading to a decrease in the evolvability of the trait. Thus, there may be a fundamental genetic limit for  $CT_{max}$  (maximum constant temperature) when measured under conditions that reflect those most closely aligned with nature, at least for knockdown resistance.

These studies challenge the notion that all traits in *D. melanogaster* are heritable and that there is no evidence of genetic limits in this species. Moreover they are consistent with limits to high thermal resistance reached in selection experiments when temperatures are rapidly increased; in these experiments, there is a selection response in initial generations but this rapidly reaches a plateau (Gilchrist and Huey, 1999; Hoffmann et al., 1997). Other physiological traits may also be close to evolutionary limits in *D. melanogaster*. In particular, acclimation ability seems to evolve with difficulty as reflected by the lack of variation in acclimation responses of populations exposed to different levels of climatic variability (Hoffmann and Watson, 1993). Desiccation resistance

also shows a signature of low heritable variation and low evolvability in some *Drosophila* species (Kellermann et al., 2006; Kellermann et al., 2009) as discussed further below.

#### Population limits: trait and gene interactions

Although trait interactions and more specifically trade-offs are often assumed to restrict selection responses in populations, there is only limited evidence from *Drosophila* studies that they constrain physiological limits. Artificial and experimental selection experiments on physiological limits have been carried out on a variety of *Drosophila* stocks and species by multiple laboratories; however, different studies often produce inconsistent patterns of correlated responses (Hoffmann et al., 2003). For instance, a negative association between cold resistance and starvation resistance detected through different approaches in one study (Hoffmann et al., 2005) was not evident in correlated responses to selection in two other studies (Bubliy and Loeschcke, 2005; MacMillan et al., 2009). Similarly, an increase in adult desiccation resistance that enhances mating success of males under high levels of desiccation stress but represents a disadvantage under mild or non-stressful conditions (Gefen and Gibbs, 2009) was not detected in earlier studies (e.g. Hoffmann and Parsons, 1993). Interactions detected between adult stress resistance and larval development as well as resistance to both these stresses and early fecundity (Mori and Kimura, 2008; Telonis-Scott et al., 2006) have turned out to be weak or undetectable in other experiments where resistance has been selected (Anderson et al., 2005; Bubliy and Loeschcke, 2005; MacMillan et al., 2009). Even when related sets of lines are exposed to the same selection pressure, patterns of correlated responses and evolutionary trajectories can be inconsistent (Simoes et al., 2008).

While the search for trait interactions has so far yielded few insights into physiological limits in populations, physiological mechanisms are nevertheless expected to produce such limits. A consistent and robust result is that increased starvation resistance in *Drosophila* is associated with an increase in fat content (Aguila et al., 2007; Ballard et al., 2008). This is expected to form the basis for correlated responses in traits linked to energy reserves including longevity or maneuverability and flight. In *Drosophila*, adult resistance to desiccation is closely tied to water-loss rates, which in turn may depend on patterns of spiracle opening and waterproofing of the cuticle (Lehmann, 2001), and these processes could influence gaseous exchange required for metabolism (Gibbs et al., 2003), although pathways of water loss in insects are still poorly resolved (Chown, 2002). Diapause is another mechanism that may underlie a robust trade-off between stress resistance and life-history traits. There is a strong cline in female reproductive diapause in *D. melanogaster* in North America, with the incidence of diapause increasing as conditions become colder; however, populations even at high latitudes tend to be polymorphic for diapause induction (Schmidt et al., 2005). This polymorphism may reflect trade-offs with life-history traits; females in diapause have a higher level of resistance to environmental stresses but also have lower fecundity (Schmidt and Paaby, 2008). However, while all of these mechanisms are expected to produce predictable patterns of correlated responses in selection experiments, one reason why consistent responses have not emerged is that several options may be available to *Drosophila* populations when evolving to shift physiological limits.

Although trait and genetic interactions have been explored through selected lines and in strain comparisons, there have been few attempts to consider the role of trait interactions in determining physiological limits in natural populations. One way of testing whether trait interactions are important in natural populations is to

consider patterns of seasonal changes in traits. In *D. serrata*, cold resistance changes seasonally at the southern (= colder) border of this species, such that higher levels of resistance are observed after winter as might be expected following selection (Jenkins and Hoffmann, 1999; Magiafoglou et al., 2002). However, there is also a loss of resistance after summer which may reflect a trade-off, most likely due to a reduction in fecundity in flies that have a relatively higher level of resistance (Jenkins and Hoffmann, 1999). Other traits influencing physiological limits also vary seasonally in a way that suggests trade-offs. This includes the incidence of reproductive diapause (Schmidt and Conde, 2006) and egg retention in virgin females (Bouletreau-Merle et al., 2003) both in *D. melanogaster*.

A difficulty in measuring trait interactions through laboratory experiments is that only a small subset of traits and conditions are usually considered in laboratory assays whereas under field conditions traits are likely to interact in complex ways. *Drosophila* data on interactions under field conditions can be generated through microcosms or by undertaking releases and measuring the success of adults in locating resources. Kristensen et al. followed this approach when they released lines selected for increased heat and cold resistance, which showed no obvious trade-offs under laboratory conditions (Kristensen et al., 2007). In field releases under hot extremes, the lines adapted to heat stress performed relatively better than controls, and under cold conditions the lines adapted to cold conditions performed relatively better; selection had therefore produced phenotypic changes likely to influence field fitness. But in addition there were also large and substantial performance costs not apparent in laboratory tests; when selected lines were released under mild conditions, both sets of lines selected for thermal tolerance had a lower probability of locating resources than controls. This suggests that traits increasing resistance under either thermal extreme may have costs under non-stressful thermal conditions.

The role of costs and trait interactions in determining physiological limits at the population level therefore remains unclear. A combination of studies is required that focuses on underlying mechanisms to allow generalisations across species, and that also addresses costs under field conditions, perhaps through release experiments as well as seasonal studies.

#### Geographical limits and gene flow

The presence of genetic variation allows for adaptive shifts in physiological limits within species in response to climatic selection and geographic variation (Table 2). There is evidence in several *Drosophila* species that such shifts have occurred. In *D. melanogaster*, a number of physiological traits related to thermal extremes including adult cold resistance and heat resistance show clinal patterns (reviewed in Hoffmann et al., 2003). There is also clinal variation in this species for the incidence of reproductive diapause (Mitrovski and Hoffmann, 2001; Schmidt et al., 2005) that correlates with levels of resistance to thermal extremes (Schmidt and Paaby, 2008). In India, highland populations of *D. melanogaster* have higher levels of desiccation resistance and melanism compared with lowland populations, which matches the hotter and drier conditions experienced in highlands (Parkash et al., 2008). Geographical patterns for stress resistance are also found in other *Drosophila* species and are usually interpreted adaptively (reviewed in Hoffmann et al., 2003). In *D. buzzatii*, altitudinal changes in cold resistance and life-history traits match larger scale latitudinal patterns and expectations based on climatic selection (Sarup et al., 2009). Levels of stress resistance may also evolve seasonally; in *D. serrata* there is a genetically based increase in cold resistance in after winter (Jenkins and Hoffmann, 1999) whereas in

*D. jambulina* melanism levels increase in response to the dry season (Parkash et al., 2009).

Adaptive responses may involve shifts in the entire thermal performance curve. Curves for viability in African *D. melanogaster* are shifted to the right of those for temperate populations (Bouletreau-Merle et al., 2003; David et al., 2004). This leads to improved performance at warm temperatures but poorer performance under cool conditions. A similar pattern is found for male sterility (David et al., 2005).

Yet in a number of cases no geographical structure has been found for physiological tolerance traits when these might be expected based on parallel clines in other species or expectations from climatic patterns. A striking example is the lack of clinal variation in *D. simulans* for stress resistance traits including cold resistance along a gradient from tropical to temperate areas despite clines along the same gradients in *D. melanogaster* (Arthur et al., 2008; David et al., 2004). Altitudinal variation in stress resistance and other traits in *D. simulans* is also less pronounced than in *D. buzzatii* (Sarup et al., 2009) whereas the viability of African populations of *D. simulans* at cool temperatures is similar to that of temperate populations, in contrast to the population differences exhibited by *D. melanogaster* (Bouletreau-Merle et al., 2003).

One reason why clines might not be apparent is that the traits scored in experiments are not under climatic selection in natural populations. Most assessments of clines in physiological traits have focused on moving adults rapidly to extremely hot or cold or dry conditions and scoring mortality or incapacitation. Traits associated with mating performance (David et al., 2005) or larval fitness (Sarup et al., 2006) are rarely scored. Ideally models need to be developed that identify patterns of climatic selection on traits along clines so that the effects of climate selection on trait distributions can be accurately predicted (Ragland and Kingsolver, 2008). The absence of spatial genetic patterns in physiological traits might also reflect trade-offs that constrain evolutionary responses. However, in practice, correlations among traits along clines can be difficult to separate from parallel patterns of selection.

Clines might also be constrained by high levels of gene flow in some species; trait clines might not develop across some gradients because strong gene flow prevents adaptive differentiation (Bridle et al., 2009a). Several models have shown that populations at species margins exposed to stressful conditions might fail to adapt because of a continual influx of genes from more central populations, particularly if the central populations are large enough in size to create asymmetrical patterns of gene flow (Bridle and Vines, 2007). While adaptive shifts in marginal populations might be prevented by gene flow, there are few empirical data from *Drosophila* that address this issue. One case where asymmetrical gene flow might play a role in physiological limits involves the southern (= high latitude) border of Australian *D. serrata*. Microsatellite data indicate that gene flow at border populations of this species is high and border populations also persist at a low density, suggesting possible gene flow from larger and denser central populations towards margins (Magiafoglou et al., 2002). Patterns of gene flow might also influence the development of clines in steep and shallow gradients in rainforest *D. birchii* (Bridle et al., 2009a). Transplant experiments provide one way of testing the role of gene flow in limiting adaptation because transplanted populations no longer experience an influx of maladapted genes from other populations. Transplant experiments have been applied to understand ecological processes in marginal plant populations (e.g. Griffith and Watson, 2006) but have not yet been used to test for physiological limits in *Drosophila* (Table 2).

The *Drosophila* studies undertaken so far indicate that clinal patterns are often found when populations are exposed to different climatic conditions. However, patterns can be non-linear (Sgro and Blows, 2003) and therefore need to be established through sampling populations along the entire cline rather than just at cline ends. They can shift seasonally (Magiafoglou et al., 2002). When clines are not detected, levels of gene flow should be examined using genetic markers like microsatellites. An absence of clinal patterns might reflect costs and constraints or else an ability of *Drosophila* to evade stressful conditions through behaviour or plastic responses.

#### Limits above the population level

Several comparisons of *Drosophila* species have been undertaken to explore links between variation in physiological traits and the distribution of species in relation to climatic variables (Table 2). These comparisons have shown that *Drosophila* distributions are closely associated with physiological limits to desiccation and cold stress (Kellermann et al., 2009; Kimura and Beppu, 1993; Parsons, 1982). Tropical species have a low level of cold and desiccation resistance, temperate species tend to be much more cold resistant, desert species are desiccation resistant and cosmopolitan species tend to have relatively high levels of resistance to most stresses. In addition, physiological limits to heat stress are related to whether species occupy exposed (open and forest canopy) or closed (understory forest) habitats (Kimura, 2004; Kimura et al., 1994). These associations persist once correlations between climatic distributions and levels of stress resistance are corrected for phylogenetic relatedness (Kimura, 2004). Patterns of diapause of *Drosophila* species may also closely match the levels of climatic extremes that species are likely to experience in nature (Kimura, 2001).

If climatic variables limit species distributions, species within an area might be expected to shift their distributions in response to changing abiotic conditions when invading new areas, as long as other factors are not of overriding importance. Perhaps the best characterised invasion for a *Drosophila* species is that of North and South America by *Drosophila subobscura* in the past few decades. This species originates in Europe where it is adapted to cool conditions and its activity is curtailed in warm and dry conditions (Huey and Pascual, 2009). Following invasion, clines along climatic gradients developed for traits and genetic polymorphisms; several clines are consistent with those found in Europe but other patterns are more complex, as in the case of desiccation resistance, which shows a consistent increase in resistance with latitude in Europe and North America but the opposite pattern in South America (Gilchrist et al., 2008). The climatic regions and latitudinal ranges occupied by this species in North and South America are similar to those found in Europe (Prevosti et al., 1988), suggesting that the invasive range of *D. subobscura* is limited by climatic variables similar to those in its native range irrespective of any changes in clinal patterns. Another invading drosophilid, *Zaprionus indianus*, introduced from tropical Africa to South America recently (Loh and Bitner-Mathe, 2005), should provide an interesting contrast because it is likely to be warm rather than cold adapted.

Although physiological limits can evolve within populations and species, there is also evidence for phylogenetic constraints on limits. For instance, Matzkin et al. found that related groups of *Drosophila* species tended to have similar levels of starvation and desiccation resistance (Matzkin et al., 2009). This meant that a significant correlation between desiccation and starvation resistance based on a comparison of species means was no longer evident once phylogenetic relationships among the species had been controlled. The extent to which thermal limits are constrained across species

is likely to depend on the nature of the trait being considered; upper thermal limits for male sterility appear to be much less constrained among related *Drosophila* species than limits for adult survival or viability (David et al., 2005). Phylogenetic constraints may lead to a mismatch between physiological limits and the climatic conditions experienced by some *Drosophila* species (Kimura, 2004).

As well as phylogenetic constraints that arise from patterns of evolution within lineages, there may also be common constraints across lineages regardless of phylogeny – as in the case of short-term heat hardening. Levels of induced (by hardening = a short sublethal exposure within the same life stage) and basal heat resistance vary among *melanogaster* and *montium* group *Drosophila* species and these do not seem to be related to their phylogeny (Kellett et al., 2005). However, there is a fundamental constraint in the level to which hardening can increase basal thermotolerance, in that induced resistance is always increased 25% over basal levels regardless of differences in resistance between species (Kellett et al., 2005). This constraint may set a limit to plastic changes in upper thermal limits.

It now appears that there is a connection between the physiological limits of species and the evolvability of populations. This finding has emerged from studies of genetic variation in desiccation and cold resistance within populations but across multiple *Drosophila* species. When variability in these traits was evaluated across rainforest species, widespread tropical species and cosmopolitan species, there was a negative association between resistance and evolvability as measured through trait heritability or the additive genetic variance (Kellermann et al., 2009). This negative association was evident even when species relationships were corrected for phylogeny. The lack of genetic variation in tropical sensitive species was not connected to an overall loss of genetic variation in these species, because tropical species from rainforests had abundant levels of microsatellite variation (van Heerwaarden et al., 2009), and there was also no difference among the species for levels of heritable variation in wing traits that were unconnected to desiccation or cold resistance (Kellermann et al., 2009). Therefore, the species characteristic of low desiccation/cold resistance was connected to an inability to extend physiological limits through evolution at the population and species levels.

At least three interpretations of such patterns are possible. One of these is that directional selection may be responsible for generating evolutionary constraints for resistance; alleles that alter resistance in one direction all become fixed in populations, decreasing genetic variation. Another possibility is that strong trait interactions are involved in generating constraints at the species level. A third possibility is that relatively sensitive species have lower levels of genetic variation for physiological traits because of a loss of genetic variation generally or DNA decay in genes controlling stress resistance when selection is relaxed for many generations.

Under directional selection, more resistant species might be expected to have lower levels of genetic variation because of directional selection on alleles that increase resistance; the more resistant species might then be expected to be approaching a fundamental physiological limit, contrary to the pattern observed. This notion of genetic variance decreasing as a fundamental limit is reached may apply to the case of heat resistance in *D. melanogaster* discussed above, because this species has a relatively high level of heat resistance compared with its relatives (Kellett et al., 2005) despite a low level of genetic variation in resistance particularly under ramping (Mitchell and Hoffmann, 2009). In the case of desiccation and cold resistance, there might be strong directional selection for decreased resistance but only if there were strong interactions among traits at the species level. This can be tested by comparing multiple traits across

species and looking for correlations; if interactions were responsible for limits across evolutionary time, some signature of these interactions might be evident across species. I am unaware of such comparisons for desiccation resistance. However, there is no evidence for interactions constraining the evolution of cold resistance; in species comparisons, thermal limits to high and low temperatures appear independent regardless of whether phylogenetic correction is carried out (Kimura, 2004).

Where genetic variance is low for relatively more sensitive species as in the case of desiccation and cold resistance, and overall levels of genetic variation are not low in these sensitive species, DNA decay may provide a plausible hypothesis for low physiological limits (Hoffmann and Willi, 2008). Under this hypothesis, historical processes have caused decay in genes that are required to evolve beyond a current limit. This occurs because when species become restricted to certain types of habitats (in this case habitats where humidity is always relatively high and temperatures are mild – as in tropical rainforests where fruit for breeding is found), there is no longer purifying selection to remove decay in genes that lead to a high level of desiccation or cold resistance. Mutations that lead to loss of function (insertions and deletions, stop codons, etc.) and then accumulate, and genes that might originally have extended physiological limits by sensing and tolerating environments are no longer active or lost (Hoffmann and Willi, 2008). This type of loss is seen in genomic comparisons of related *Drosophila* species that use different hosts for breeding, where specialisation on one host plant has led to decay in genes that are associated with responding to other hosts as reflected by null alleles, the evolution of pseudogenes and loss of genes (McBride, 2007).

#### Limits and climate change

Can the physiological limits of *Drosophila* species provide information on their relative level of susceptibility to the effects of climate change? Deutsch et al. proposed a framework for evaluating susceptibility based on the thermal response curve of organisms (Deutsch et al., 2008). They collated data from mostly pest insects where species had been cultured under a range of constant temperatures and used this to define the  $CT_{max}$  and  $CT_{min}$  (minimum constant temperature) of a species as well as optimal conditions by assuming that performance curves for standardised growth rate followed a consistent relationship across species. The resulting  $CT_{max}$  was then compared with ambient conditions experienced in the insect's environments to determine susceptibility to current and future conditions. The susceptibility of species to climate change was dictated by distance from  $CT_{max}$  to thermal averages and the degree of seasonality in the environment. This analysis indicated that tropical species were currently closer to limits and had smaller safety margins than temperate species, and that this situation would persist under climate change predictions.

These analyses considered only one *Drosophila* species but the approach can be applied more widely to *Drosophila*. As indicated previously, *Drosophila* species distributions are often associated closely with physiological limits under extreme conditions and limits for culturing flies and when fertility can be maintained (David et al., 2005; Gibert et al., 2001; Kellermann et al., 2009; Kimura et al., 1994; Parsons, 1982). Thus, desiccation and cold resistance levels of species when scored in standardised ways are directly linked to the climates from which species originate; tropical species are highly sensitive to both stresses, cold adapted species are resistant to cold but moderately sensitive to desiccation and desert species are highly resistant to desiccation (Gibert et al., 2001; Kellermann et al., 2009).

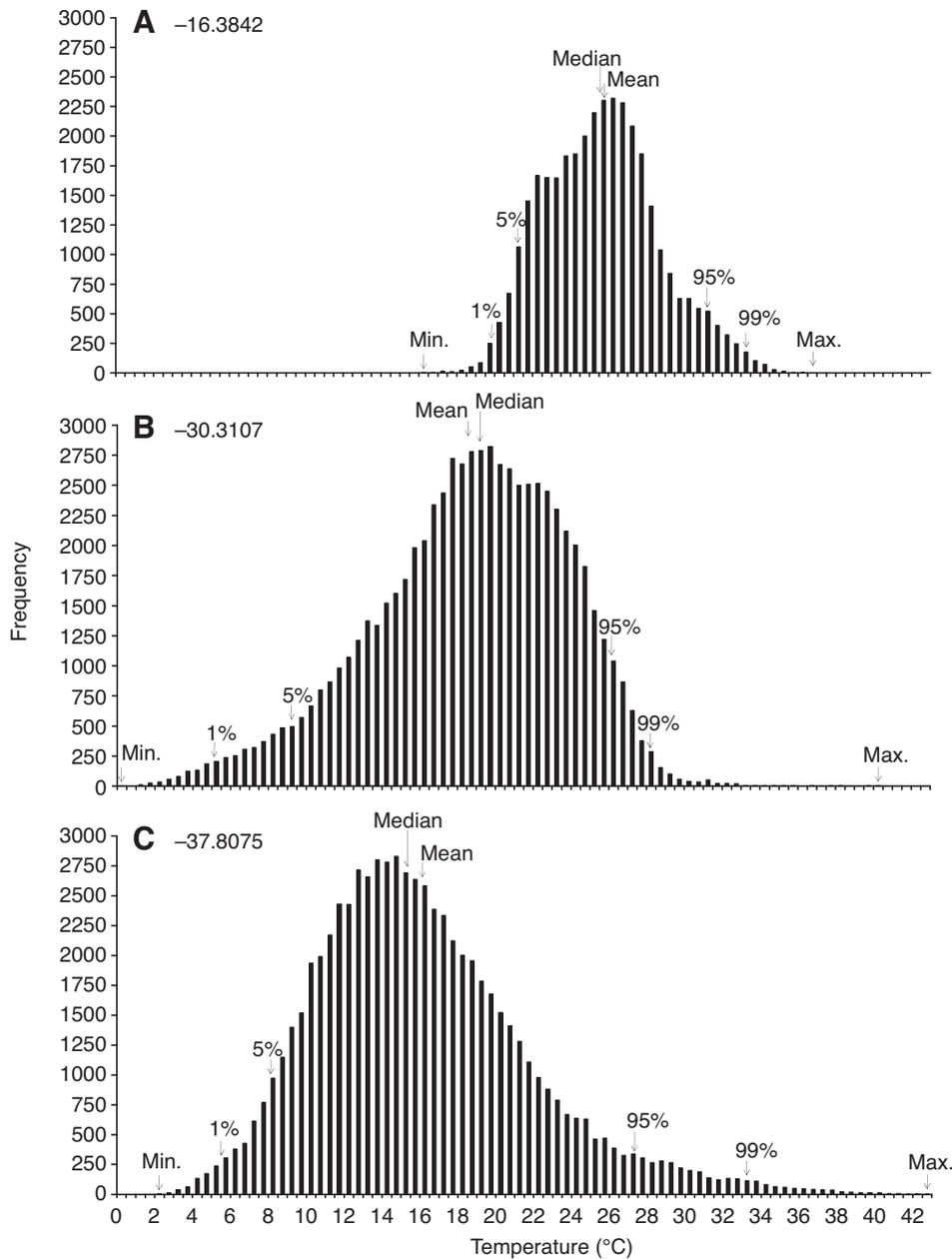


Fig. 2. Distribution of temperatures in (A) tropical, (B) subtropical and (C) temperate locations from the east coast of Australia. Data are based on three-hourly records from 2005 to 2008 placed into 0.5°C categories from low altitude (<100 m ASL). Locations derived from www.bom.gov.au.

Culture temperature data for *Drosophila* species suggest that there are only minor differences in upper culture temperatures between tropical and temperate species, being at around 31–32°C whereas lower thermal responses vary quite markedly between 6°C and 17°C with tropical species at the higher end of this range (Parkash and Munjal, 1999). Given the higher average field temperatures experienced by *Drosophila* in the tropics compared with temperate areas, this may place tropical species closer to culture limits both currently and into the future. In terms of the terminology used in Deutsch et al. (Deutsch et al., 2008), tropical species have a smaller thermal safety margin than temperate species.

However, the analysis of Deutsch et al. (Deutsch et al., 2008) is based on average conditions experienced in the different environments rather than the extreme conditions that species encounter for periods of a few hours or less. Small adult flies like *Drosophila* are likely to have body temperatures that approach ambient conditions although they can behaviourally thermoregulate

to some extent by moving to shady areas. If the distribution of ambient temperatures in the shade is plotted for three locations from three-hourly readings at locations on the east coast of Australia from a tropical and temperate location as well as a mid-latitude location, it is apparent that average conditions do not necessarily reflect extremes (Fig. 2). The right-hand distribution tail is much shorter in the tropical location than in the temperate one. This leads to higher extremes of hot and cold conditions being recorded in the southerly temperate location. The top 1% of readings are the same in this location as in the tropics. Because groups of tropical and temperate species tend to have similar levels of heat resistance (Kimura, 2004) while differing markedly in terms of cold resistance, this means that tropical populations are likely to be less exposed to short periods of very high temperatures at the limits of their tolerance (Fig. 1). This suggests that the safety margin will be less for temperate species when short, extremely stressful, periods are considered rather than average conditions as in Deutsch et al. (Deutsch et al., 2008). The

*Drosophila* data suggest that tropical species might not necessarily be more susceptible to extinction than temperate species under climate change, particularly as temperature increases are expected to be greater at higher latitudes than at lower ones.

The *Drosophila* results also emphasise that, as well as thermal extremes, aridity responses need to be considered when evaluating the relative susceptibility of species to climate change. Although predictions about future changes in humidity and rainfall are less clear-cut than predictions about temperature (www.ipcc.ch), rainfall patterns are nevertheless expected to change regionally, and species in many tropical regions are expected to experience drier conditions. Fragmentation and deforestation is further likely to contribute to the development of drier conditions (Nair et al., 2003). Tropical species with a low level of resistance to desiccation and a very limited evolutionary potential to further increase physiological limits may be particularly prone to drier conditions.

Finally, the *Drosophila* data highlight that diapause needs to be considered when predicting population and species responses to climate change. By entering reproductive or immature diapause, species can evade stressful conditions and increase their resistance not only to cold conditions but also to heat and drought. *Drosophila* populations are often polymorphic for diapause and it is possible that rapid evolutionary shifts in the incidence and nature of diapause induction as already documented for pitcher plant mosquitoes (Bradshaw and Holzapfel, 2007) may help to extend physiological limits of *Drosophila* species.

### Concluding remarks

The above discussion highlights some of the advantages and disadvantages of using *Drosophila* when investigating physiological limits. On the one hand, there is excellent background information on species taxonomy, and many species are easy to rear, allowing for detailed quantitative genetic studies, and allowing for multi-generation experiments across species where conditions can be precisely controlled. *Drosophila* studies also allow for detailed analysis studies of physiological limits, and it is becoming possible to make genetic comparisons across species as more genomes are sequenced. On the other hand, information on the distribution of many *Drosophila* species is currently rudimentary and there is often also only limited information on larval breeding sites, which can make it difficult to assess the physiological stresses to which different life stages are exposed. It is also quite difficult to track *Drosophila* in the field, which would help when assessing stress levels. There are some core issues around physiological limits for which we currently have limited data in *Drosophila* and other insects; these include the impact of multiple stressors and biotic interactions on physiological limits, the impact of extremes on limits and species distributions and the effect of gene flow on geographical limits. Additional studies across *Drosophila* species that are found in a variety of climatic regions could assist in providing information on these issues.

### Acknowledgements

A.A.H.'s research is supported by the Australian Research Council (Discovery, Federation Fellowship) and the Commonwealth Environment Research Facilities. He thanks Steven Chown and an anonymous reviewer for comments on the manuscript.

### References

- Aguila, J. R., Suszko, J., Gibbs, A. G. and Hoshizaki, D. K. (2007). The role of larval fat cells in adult *Drosophila melanogaster*. *J. Exp. Biol.* **210**, 956-963.
- Anderson, A. R., Hoffmann, A. A. and McKechnie, S. W. (2005). Response to selection for rapid chill-coma recovery in *Drosophila melanogaster*: physiology and life-history traits. *Gene. Res.* **85**, 15-22.
- Angilletta, M. J. (2009). *Thermal Adaptation*. New York: Oxford University Press.
- Arthur, A. L., Weeks, A. R. and Sgro, C. M. (2008). Investigating latitudinal clines for life history and stress resistance traits in *Drosophila simulans* from eastern Australia. *J. Evol. Biol.* **21**, 1470-1479.
- Ballard, J. W. O., Melvin, R. G. and Simpson, S. J. (2008). Starvation resistance is positively correlated with body lipid proportion in five wild caught *Drosophila simulans* populations. *J. Insect Physiol.* **54**, 1371-1376.
- Bernardo, J. and Spotila, J. R. (2006). Physiological constraints on organismal response to global warming: mechanistic insights from clinally varying populations and implications for assessing endangerment. *Biol. Lett.* **2**, 135-139.
- Bernardo, J., Ossola, R. J., Spotila, J. and Crandall, K. A. (2007). Interspecies physiological variation as a tool for cross-species assessments of global warming-induced endangerment: validation of an intrinsic determinant of macroecological and phylogeographic structure. *Biol. Lett.* **3**, 695-698.
- Besansky, N. J., Krzywinski, J., Lehmann, T., Simard, F., Kern, M., Mukabayire, O., Fontenille, D., Toure, Y. and Sagnon, N. F. (2003). Semipermeable species boundaries between *Anopheles gambiae* and *Anopheles arabiensis*: evidence from multilocus DNA sequence variation. *Proc. Nat. Acad. Sci. USA* **100**, 10818-10823.
- Blows, M. W. (2007). A tale of two matrices: multivariate approaches in evolutionary biology. *J. Evol. Biol.* **20**, 1-8.
- Blows, M. W. and Hoffmann, A. A. (2005). A reassessment of genetic limits to evolutionary change. *Ecol.* **86**, 1371-1384.
- Bouletreau-Merle, J., Fouillet, P. and Varaldi, J. (2003). Divergent strategies in low temperature environment for the sibling species *Drosophila melanogaster* and *D. simulans*: overwintering in extension border areas of France and comparison with African populations. *Evol. Ecol.* **17**, 523-548.
- Bradshaw, A. D. (1991). The Croonian lecture, 1991 – Genostasis and the limits to evolution. *Phil. Trans. R. Soc. London Series B Biol. Sci.* **333**, 289-305.
- Bradshaw, W. E. and Holzapfel, C. M. (2007). Genetic response to rapid climate change: it's seasonal timing that matters. *Mol. Ecol.* **17**, 157-166.
- Bridle, J. R. and Vines, T. H. (2007). Limits to evolution at range margins: when and why does adaptation fail? *Trends. Ecol. Evol.* **22**, 140-147.
- Bridle, J. R., Gavaz, S. and Kennington, W. J. (2009a). Testing limits to adaptation along altitudinal gradients in rainforest *Drosophila*. *Proc. R. Soc. B Biol. Sci.* **276**, 1507-1515.
- Bridle, J. R., Polechova, J. and Vines, T. H. (2009b). Limits to adaptation and patterns of biodiversity. In *Speciation and Patterns of Diversity* (eds R. Butlin J. Bridle and D. Schluter). Cambridge: Cambridge University Press.
- Bubliy, O. A. and Loeschcke, V. (2005). Correlated responses to selection for stress resistance and longevity in a laboratory population of *Drosophila melanogaster*. *J. Evol. Biol.* **18**, 789-803.
- Burger, R. and Lynch, M. (1995). Evolution and extinction in a changing environment – a quantitative-genetic analysis. *Evolution* **49**, 151-163.
- Burke, M. K. and Rose, M. R. (2009). Experimental evolution with *Drosophila*. *Am. J. Physiol. Reg. Integr. Comp. Physiol.* **296**, R1847-R1854.
- Chakir, M., Chafik, A., Moreteau, B., Gibert, P. and David, J. R. (2002). Male sterility thermal thresholds in *Drosophila*: *D. simulans* appears more cold-adapted than its sibling *D. melanogaster*. *Genetica* **114**, 195-205.
- Chown, S. L. (2002). Respiratory water loss in insects. *Comp. Biochem. Physiol. Mol. Inter. Physiol.* **133**, 791-804.
- Chown, S. L., Jumbam, K. R., Sorensen, J. G. and Terblanche, J. S. (2009). Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Funct. Ecol.* **23**, 133-140.
- Czajka, M. C. and Lee, R. E. (1990). A rapid cold-hardening response protecting against cold shock injury in *Drosophila melanogaster*. *J. Exp. Biol.* **148**, 245-254.
- David, J. R., Allemand, R., Capy, P., Chakir, M., Gibert, P., Petavy, G. and Moreteau, B. (2004). Comparative life histories and ecophysiology of *Drosophila melanogaster* and *D. simulans*. *Genetica* **120**, 151-163.
- David, J. R., Araripe, L. O., Chakir, M., Legout, H., Lemos, B., Petavy, G., Rohmer, C., Joly, D. and Moreteau, B. (2005). Male sterility at extreme temperatures: a significant but neglected phenomenon for understanding *Drosophila* climatic adaptations. *J. Evol. Biol.* **90**, 838-846.
- Davis, A. J., Lawton, J. H., Shorrocks, B. and Jenkinson, L. S. (1998). Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. *J. Anim. Ecol.* **67**, 600-612.
- Denny, M. W., Miller, L. P. and Harley, C. D. G. (2006). Thermal stress on intertidal limpets: long-term hindcasts and lethal limits. *J. Exp. Biol.* **209**, 2420-2431.
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C. and Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Nat. Acad. Sci. USA* **105**, 6668-6672.
- Dillon, M. E., Wang, G., Garrity, P. A. and Huey, R. B. (2009). Thermal preference in *Drosophila*. *J. Therm. Biol.* **34**, 109-119.
- Duncan, R. P., Cassey, P. and Blackburn, T. M. (2009). Do climate envelope models transfer? A manipulative test using dung beetle introductions. *Proc. R. Soc. B Biol. Sci.* **276**, 1449-1457.
- Elton, C. (1930). *Animal Ecology and Evolution*. Oxford: Clarendon Press.
- Feder, M. E., Blair, N. and Figuras, H. (1997). Natural thermal stress and heat-shock protein expression in *Drosophila* larvae and pupae. *Funct. Ecol.* **11**, 90-100.
- Feder, M. E., Roberts, S. P. and Bordeleon, A. C. (2000). Molecular thermal telemetry of free-ranging adult *Drosophila melanogaster*. *Oecologia* **123**, 460-465.
- Flint, J. and Mackay, T. F. C. (2009). Genetic architecture of quantitative traits in mice, flies, and humans. *Genome Research* **19**, 723-733.
- Gefen, E. and Gibbs, A. G. (2009). Interactions between environmental stress and male mating success may enhance evolutionary divergence of stress-resistant *Drosophila* populations. *Evolution* **63**, 1653-1659.
- Gibbs, A. G. (1999). Laboratory selection for the comparative physiologist. *J. Exp. Biol.* **202**, 2709-2718.

- Gibbs, A. G., Louie, A. K. and Ayala, J. A. (1998). Effects of temperature on cuticular lipids and water balance in a desert *Drosophila*: is thermal acclimation beneficial? *J. Exp. Biol.* **201**, 71-80.
- Gibbs, A. G., Fukuzato, F. and Matzkin, L. M. (2003). Evolution of water conservation mechanisms in *Drosophila*. *J. Exp. Biol.* **206**, 1183-1192.
- Gibert, P., Moreteau, B., Petavy, G., Karan, D. and David, J. R. (2001). Chill-coma tolerance, a major climatic adaptation among *Drosophila* species. *Evolution* **55**, 1063-1068.
- Gilchrist, G. W. (1995). Specialists and generalists in changing environments. 1. Fitness landscapes of thermal sensitivity. *Am. Nat.* **146**, 252-270.
- Gilchrist, G. W. and Huey, R. B. (1999). The direct response of *Drosophila melanogaster* to selection on knockdown temperature. *Heredity* **83**, 15-29.
- Gilchrist, G. W., Jeffers, L. M., West, B., Folk, D. G., Suess, J. and Huey, R. B. (2008). Clinal patterns of desiccation and starvation resistance in ancestral and invading populations of *Drosophila subobscura*. *Evol. Appl.* **1**, 513-523.
- Griffith, T. M. and Watson, M. A. (2006). Is evolution necessary for range expansion? Manipulating reproductive timing of a weedy annual transplanted beyond its range. *Am. Nat.* **167**, 153-164.
- Hansen, T. F. and Houle, D. (2004). Evolvability, stabilizing selection, and the problem of stasis. In *Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes* (eds M. Pigliucci and K. Preston), pp. 130-150. Oxford: Oxford University Press.
- Helmuth, B. (2009). From cells to coastlines: how can we use physiology to forecast the impacts of climate change? *J. Exp. Biol.* **212**, 753-760.
- Hoffmann, A. A. (1990). Acclimation for desiccation resistance in *Drosophila melanogaster* and the association between acclimation responses and genetic variation. *J. Insect Physiol.* **36**, 885-891.
- Hoffmann, A. A. and Parsons, P. A. (1991). *Evolutionary Genetics and Environmental Stress*. Oxford: Oxford University Press.
- Hoffmann, A. A. and Parsons, P. A. (1993). Selection for adult desiccation resistance in *Drosophila melanogaster*-fitness components, larval resistance and stress correlations. *Biol. J. Linn. Soc.* **48**, 43-54.
- Hoffmann, A. A. and Watson, M. (1993). Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. *Amer. Nat.* **142**, S93-S113.
- Hoffmann, A. A. and Willi, Y. (2008). Detecting genetic responses to environmental change. *Nat. Rev. Genet.* **9**, 421-432.
- Hoffmann, A. A., Dagher, H., Hercus, M. and Berrigan, D. (1997). Comparing different measures of heat resistance in selected lines of *Drosophila melanogaster*. *J. Insect Physiol.* **43**, 393-405.
- Hoffmann, A. A., Anderson, A. and Hallas, R. (2002). Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecol. Lett.* **5**, 614-618.
- Hoffmann, A. A., Sorensen, J. G. and Loeschcke, V. (2003). Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Therm. Biol.* **28**, 175-216.
- Hoffmann, A. A., Hallas, R., Anderson, A. R. and Telonis-Scott, M. (2005). Evidence for a robust sex-specific trade-off between cold resistance and starvation resistance in *Drosophila melanogaster*. *J. Evol. Biol.* **18**, 804-810.
- Holt, R. D. and Gaines, M. S. (1992). Analysis of adaptation in heterogeneous landscapes-implications for the evolution of fundamental niches. *Evol. Ecol.* **6**, 433-447.
- Huey, R. and Kingsolver, J. (1989). Evolution of thermal sensitivity of ectotherm performance. *Trends Ecol. Evol.* **4**, 131-135.
- Huey, R. B. and Pascual, M. (2009). Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline. *Ecology* **90**, 1715-1720.
- Huey, R. B., Hertz, P. E. and Sinervo, B. (2003). Behavioral drive versus behavioral inertia in evolution: a null model approach. *Am. Nat.* **161**, 357-366.
- Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Perez, H. J. A. and Garland, T. (2009). Why tropical forest lizards are vulnerable to climate warming. *Proc. R. Soc. B Biol. Sci.* **276**, 1939-1948.
- Jenkins, N. L. and Hoffmann, A. A. (1999). Limits to the southern border of *Drosophila serrata*: Cold resistance, heritable variation, and trade-offs. *Evolution* **53**, 1823-1834.
- Kellermann, V. M., Van Heerwaarden, B., Hoffmann, A. A. and Sgro, C. M. (2006). Very low additive genetic variance and evolutionary potential in multiple populations of two rainforest *Drosophila* species. *Evolution* **60**, 1104-1108.
- Kellermann, V. M., van Heerwaarden, B., Sgrò, C. M. and Hoffmann, A. A. (2009). Fundamental evolutionary limits in ecological traits drive *Drosophila* species distributions. *Science* **325**, 1244-1246.
- Kellett, M., Hoffmann, A. A. and McKechnie, S. W. (2005). Hardening capacity in the *Drosophila melanogaster* species group is constrained by basal thermotolerance. *Funct. Ecol.* **19**, 853-858.
- Kelty, J. D. and Lee, R. E. (1999). Induction of rapid cold hardening by cooling at ecologically relevant rates in *Drosophila melanogaster*. *J. Insect Physiol.* **45**, 719-726.
- Kimura, M. (2001). Evolutionary aspects of photoperiodism in *Drosophila*. In *Insect Timing: Circadian Rhythmicity to Seasonality* (eds D. Denlinger, J. Giebultowicz and D. Saunders), pp. 123-132. Amsterdam: Elsevier.
- Kimura, M. T. (2004). Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia* **140**, 442-449.
- Kimura, M. T. and Beppu, K. (1993). Climatic adaptations in the *Drosophila immigrans* species group-seasonal migration and thermal tolerance. *Ecol. Entomol.* **18**, 141-149.
- Kimura, M. T., Ohtsu, T., Yoshida, T., Awasaki, T. and Lin, F. J. (1994). Climatic adaptations and distributions in the *Drosophila takahashii* species subgroup (Diptera: Drosophilidae). *J. Nat. Hist.* **28**, 401-409.
- Kirkpatrick, M. and Barton, N. H. (1997). Evolution of a species' range. *Am. Nat.* **150**, 1-23.
- Krebs, R. A. and Loeschcke, V. (1994). Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster*. *Funct. Ecol.* **8**, 730-737.
- Kristensen, T. N., Loeschcke, V. and Hoffmann, A. A. (2007). Can artificially selected phenotypes influence a component of field fitness? Thermal selection and fly performance under thermal extremes. *Proc. R. Soc. B Biol. Sci.* **274**, 771-778.
- Lehmann, F. A. (2001). Matching spiracle opening to metabolic need during flight in *Drosophila*. *Science* **294**, 1926-1929.
- Loeschcke, V. and Hoffmann, A. A. (2007). Consequences of heat hardening on a field fitness component in *Drosophila* depend on environmental temperature. *Am. Nat.* **169**, 175-183.
- Loh, R. and Bitner-Mathe, B. (2005). Variability of wing size and shape in three populations of a recent Brazilian invader, *Zaprionus indianus* (Diptera: Drosophilidae), from different habitats. *Genetica* **125**, 271-281.
- Lynch, M. and Lande, R. (1993). Evolution and extinction in response to environmental change. In *Biotic Interactions and Global Change* (eds P. Kareiva, J. Kingsolver and R. B. Huey). Sunderland, Mass: Sinauer.
- MacMillan, H. A., Walsh, J. P. and Sinclair, B. J. (2009). The effects of selection for cold tolerance on cross-tolerance to other environmental stressors in *Drosophila melanogaster*. *Insect Sci.* **16**, 263-276.
- Magiafoglou, A., Carew, M. E. and Hoffmann, A. A. (2002). Shifting clinal patterns and microsatellite variation in *Drosophila serrata* populations: a comparison of populations near the southern border of the species range. *J. Evol. Biol.* **15**, 763-774.
- Marais, E. and Chown, S. L. (2008). Beneficial acclimation and the Bogert effect. *Ecol. Lett.* **11**, 1027-1036.
- Matzkin, L. M., Watts, T. D. and Markow, T. A. (2009). Evolution of stress resistance in *Drosophila*: interspecific variation in tolerance to desiccation and starvation. *Funct. Ecol.* **23**, 521-527.
- McBride, C. S. (2007). Rapid evolution of smell and taste receptor genes during host specialization. *Proc. Natl. Acad. Sci. USA* **104**, 4996-5001.
- McCull, G., Hoffmann, A. A. and McKechnie, S. W. (1996). Response of two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*. *Genetics* **143**, 1615-1627.
- Merila, J., Sheldon, B. C. and Kruuk, L. E. B. (2001). Explaining stasis: microevolutionary studies in natural populations. *Genetica* **112**, 199-222.
- Mitchell, K. A. and Hoffmann, A. A. (2010). Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in *Drosophila*. *Funct. Ecol.* doi: 10.1111/j.1365-2435.2009.01666.x
- Mitrovski, P. and Hoffmann, A. A. (2001). Postponed reproduction as an adaptation to winter conditions in *Drosophila melanogaster*: evidence for clinal variation under semi-natural conditions. *Proc. R. Soc. Lond. Series B Biol. Sci.* **268**, 2163-2168.
- Mori, N. and Kimura, M. T. (2008). Selection for rapid and slow recovery from chill- and heat-coma in *Drosophila melanogaster*. *Biol. J. Linn. Soc.* **95**, 72-80.
- Nair, U. S., Lawton, R. O., Welch, R. M. and Pielke, R. A. (2003). Impact of land use on Costa Rican tropical montane cloud forests: Sensitivity of cumulus cloud field characteristics to lowland deforestation. *J. Geophys. Res. Atmos.* **108**, 13.
- Parkash, R. and Munjal, A. K. (1999). Climatic selection of starvation and desiccation resistance in populations of some tropical drosophilids. *J. Zool. Syst. Evol. Res.* **37**, 195-202.
- Parkash, R., Rajpurohit, S. and Ramniwas, S. (2008). Changes in body melanisation and desiccation resistance in highland vs. lowland populations of *D. melanogaster*. *J. Insect Physiol.* **54**, 1050-1056.
- Parkash, R., Singh, S. and Ramniwas, S. (2009). Seasonal changes in humidity level in the tropics impact body color polymorphism and desiccation resistance in *Drosophila jambulina* - evidence for melanism-desiccation hypothesis. *J. Insect Physiol.* **55**, 358-368.
- Parsons, P. A. (1982). Evolutionary ecology of Australian *Drosophila* - a species analysis. *Evol. Biol.* **14**, 297-350.
- Petavy, G., David, J. R., Gibert, P. and Moreteau, B. (2001). Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. *J. Therm. Biol.* **26**, 29-39.
- Prevosti, A., Ribo, G., Serra, L., Aguade, M., Balana, J., Monclus, M. and Mestres, F. (1988). Colonization of America by *Drosophila subobscura* - experiment in natural-populations that supports the adaptive role of chromosomal-inversion polymorphism. *Proc. Natl. Acad. Sci. USA* **85**, 5597-5600.
- Prince, G. J. and Parsons, P. A. (1977). Adaptive behavior of *Drosophila* adults in relation to temperature and humidity. *Aus. J. Zool.* **25**, 285-290.
- Ragland, G. J. and Kingsolver, J. G. (2008). Evolution of thermotolerance in seasonal environments: The effects of annual temperature variation and life-history timing in *Wyeomyia smithii*. *Evolution* **62**, 1345-1357.
- Rako, L. and Hoffmann, A. A. (2006). Complexity of the cold acclimation response in *Drosophila melanogaster*. *J. Insect Physiol.* **52**, 94-104.
- Sarup, P., Sorensen, J. G., Dimitrov, K., Barker, J. S. F. and Loeschcke, V. (2006). Climatic adaptation of *Drosophila buzzatii* populations in southeast Australia. *Heredity* **96**, 479-486.
- Sarup, P., Frydenberg, J. and Loeschcke, V. (2009). Local adaptation of stress related traits in *Drosophila buzzatii* and *Drosophila simulans* in spite of high gene flow. *J. Evol. Biol.* **22**, 1111-1122.
- Schmidt, P. S. and Conde, D. R. (2006). Environmental heterogeneity and the maintenance of genetic variation for reproductive diapause in *Drosophila melanogaster*. *Evolution* **60**, 1602-1611.
- Schmidt, P. S. and Paaby, A. B. (2008). Reproductive diapause and life-history clines in North American populations of *Drosophila melanogaster*. *Evolution* **62**, 1204-1215.
- Schmidt, P. S., Matzkin, L., Ippolito, M. and Eanes, W. F. (2005). Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. *Evolution* **59**, 1721-1732.
- Sgrò, C. M. and Blows, M. W. (2003). Evolution of additive and nonadditive genetic variance in development time along a cline in *Drosophila serrata*. *Evolution* **57**, 1846-1851.
- Simoes, P., Santos, J., Fragata, I., Mueller, L. D., Rose, M. R. and Matos, M. (2008). How repeatable is adaptive evolution? The role of geographical origin and founder effects in laboratory adaptation. *Evolution* **62**, 1817-1829.

- Sorensen, J. G., Michalak, P., Justesen, J. and Loeschcke, V. (1999). Expression of the heat-shock protein HSP70 in *Drosophila buzzatii* lines selected for thermal resistance. *Hereditas* **131**, 155-164.
- Telonis-Scott, M., Guthridge, K. M. and Hoffmann, A. A. (2006). A new set of laboratory-selected *Drosophila melanogaster* lines for the analysis of desiccation resistance: response to selection, physiology and correlated responses. *J. Exp. Biol.* **209**, 1837-1847.
- Terblanche, J. S., Deere, J. A., Clusella-Trullas, S., Janion, C. and Chown, S. L. (2007). Critical thermal limits depend on methodological context. *Proc. R. Soc. B Biol. Sci.* **274**, 2935-2942.
- van Heerwaarden, B., Kellermann, V., Schiffer, M., Blacket, M., Sgro, C. M. and Hoffmann, A. A. (2009). Testing evolutionary hypotheses about species borders: patterns of genetic variation towards the southern borders of two rainforest *Drosophila* and a related habitat generalist. *Proc. R. Soc. B Biol. Sci.* **276**, 1517-1526.
- Wiens, J. J. and Graham, C. H. (2005). Niche conservatism: integrating evolution, ecology, and conservation biology. *Ann. Rev. Ecol. Syst.* **36**, 519-539.
- Willi, Y. and Hoffmann, A. A. (2009). Demographic factors and genetic variation influence population persistence under environmental change. *J. Evol. Biol.* **22**, 124-133.
- Willi, Y., Van Buskirk, J. and Hoffmann, A. A. (2006). Limits to the adaptive potential of small populations. *Ann. Rev. Ecol. Syst.* **37**, 433-458.
- Williams, S. E., Shoo, L. P., Isaac, J. L., Hoffmann, A. A. and Langham, G. (2008). Towards an integrated framework for assessing the vulnerability of species to climate change. *Plos Biol.* **6**, 2621-2626.
- Wilson, A. J., Pemberton, J. M., Pilkington, J. G., Coltman, D. W., Mifsud, D. V., Clutton-Brock, T. H. and Kruuk, L. E. B. (2006). Environmental coupling of selection and heritability limits evolution. *Plos Biol.* **4**, 1270-1275.
- Zhang, X. S. and Hill, W. G. (2005). Genetic variability under mutation selection balance. *Trend. Ecol. Evol.* **20**, 468-470.