REVIEW

CENTENARY ARTICLE

100 YEARS OF DISCOVERY

Testing evolutionary adaptation potential under climate change in invertebrates (mostly *Drosophila*): findings, limitations and directions

Ary A. Hoffmann[1],*, Carla M. Sgrò[2] and Belinda van Heerwaarden[1]

ABSTRACT

A (quite) large set of experiments has been undertaken to assess the potential for evolutionary changes in invertebrates under current and future climate change conditions. These experimental studies have established some key principles that could affect climate change adaptation, yet there remain substantial obstacles in reaching a meaningful predictive framework. This Review starts with exploring some of the traits considered in individuals and approaches used in assessing evolutionary adaptation relevant to climate, and some of the core findings and their substantial limitations, with a focus on *Drosophila*. We interpret results in terms of adaptive limits based on population processes versus fundamental mechanistic limits of organisms. We then consider the challenges in moving towards a predictive framework and implications of the findings obtained to date, while also emphasizing the current limited context and the need to broaden it if links to changes in natural populations are to be realized.

KEY WORDS: Adaptation, Climate, Evolution, Invertebrate, Prediction

Introduction

Current and projected changes in climate pose a major risk to species persistence and invertebrate biodiversity. Understanding which invertebrate groups and regions are particularly sensitive to climate change (Deutsch et al., 2008; Jørgensen et al., 2022), and their ability to evolve to keep pace with these changes, will be crucial for predicting and managing the impacts of anthropogenic effects. Research on climate change adaptation in invertebrates has expanded massively recently, as the early effects of this phenomenon are impacting the distribution and phenotypes of species. Some examples involving invertebrates include phenological changes in the timing of diapause, flight and other key developmental shifts (Bradshaw and Holzapfel, 2008; Robinet and Roques, 2010), the development of multivoltine life cycles (see Glossary; Altermatt, 2010; Herremans et al., 2021), distribution shifts of both marine and terrestrial invertebrates (Hiddink et al., 2015; Netherer and Schopf, 2010) and both physiological and morphological changes in insects (Clusella-Trullas and Nielsen, 2020).

Evolutionary shifts in traits under climate change are starting to be documented, including those involving phenology (Bradshaw and Holzapfel, 2008; Singer and Parmesan, 2010) and morphology

[1]PEARG group, Bio21 Institute, School of BioSciences, The University of Melbourne, Parkville, VIC 3010, Australia. [2]Department of Biological Sciences, Monash University, Clayton, VIC 3800, Australia.

*Author for correspondence (ary@unimelb.edu.au)

A.A.H., 0000-0001-9497-7645

(Nielsen and Kingsolver, 2020; Zvereva et al., 2019). There are few examples of evolutionary changes in tolerance to thermal (or aridity) conditions; inherent interspecific differences in responses to these factors correlate closely with tolerance levels in insects, including *Drosophila*, implying past evolved changes in tolerance producing differences among species (Kellermann et al., 2012; Parratt et al., 2021).

One challenge in testing for responses to climate change is that many environmental components are changing, and it is difficult to capture these experimentally (Fig. 1). Although it is possible to test for increases in average temperature or even the incidence of thermal extremes, it is another matter to capture the complexity of changes in microclimate, biotic interactions, behavioural modification and diapause responses affecting organisms. In community ecology, experiments can capture complex environments; for example, using terraria heated by an infrared lamp in natural vegetation, and in which predator–prey interactions are monitored (e.g. Barton and Schmitz, 2009). But, even here, long-term monitoring is required to capture rare events – such as extended periods of drought – that are critically important in community-level changes (Thompson et al., 2013). Spatial gradients in climate may serve as a proxy for changes across time when making predictions, but the correlation between changes in communities across gradients and those seen in experimentally warmed plots can be poor (Menke et al., 2014).

Because evolutionary changes in invertebrates are enmeshed within this complex of biotic and microenvironmental changes where the effects of selection accumulate across multiple generations, evolutionary responses are inherently difficult to measure and predict. Yet, most invertebrate studies have focused on responses to individual environmental components, limited mainly to temperature and to a lesser extent humidity, with only a handful of studies considering CO_2. Few experimental studies have allowed for behavioural adaptation, life-history impacts, biotic interactions and so on (Kingsolver and Buckley, 2020). Responses to abiotic factors can depend on plastic changes, and these have also rarely been considered across species life cycles (Hoffmann and Bridle, 2022; Sgrò et al., 2016).

In this Review, we start by discussing the importance of distinguishing between adaptation limited by population processes (soft) versus fundamental (hard) limits and highlighting key factors and complexities when assessing evolutionary adaptation to climate change. We then outline core findings and limitations from past studies and consider the challenges in moving towards a predictive framework for assessing adaptive responses and vulnerability.

Population (soft) versus fundamental (hard) limits

A starting point in understanding evolutionary adaptation to climate change is to distinguish between adaptation limited by population processes (soft) versus fundamental (hard) limits (Antonovics,

Glossary

Adaptive tracking
Continuous genetic adaptation in response to rapid environmental change.

Broad sense heritability
The fraction of phenotypic variance of a trait that can be attributed to total genotypic variance, which includes additive genetic variance, as well as dominance and epistatic variance.

Evolve and re-sequence studies
The combination of experimental evolution with whole-genome sequencing of pooled (groups) of individuals, aimed at identifying the genomic basis of evolutionary changes.

Extinction temperatures
Temperatures at which populations can no longer persist, leading to extinction.

Family studies
Trait data from related individuals are used to quantify the extent to which genetic factors contribute to trait (phenotypic) variation within populations. They can be used to estimate heritability and evolutionary adaptive potential.

Field cages
Cages that are placed in the field to simulate natural conditions, while allowing population abundance to be recorded. Cages can vary in size, and resources are usually provided, so populations are assumed to be largely responding to changes in natural thermal conditions, rather than other factors that determine population growth.

Genetic redundancy
Two or more genes perform the same function and inactivation of one of these genes has little effect on the trait/function in question.

Hard limits
Limits to evolutionary adaptation that reflect inherent limits such as an absence of genetic variation or trait thresholds that cannot easily be overcome without the spread of novel of rare mutations that will alter existing phenotypic variation or developmental/ecological trade-offs.

Hardening
Plastic response to short-term (minutes, hours) exposure to a sublethal thermal stress.

Heat knock-down time
The time taken for an individual to become unresponsive after exposure to a heat stress, usually using a static assay.

Introgression
The transfer of genetic material from one species into the gene pool of another.

Jensen's inequality (as applied to thermal performance curves)
The inability to describe the thermal performance of an organism or population based on average temperature because of the non-linear association between temperature and performance (with performance dropping particularly steeply at high temperatures).

Lethal thermal limits
The upper or lower temperatures at which survival is no longer possible.

Multivoltine life cycle
Life cycle of invertebrate species that can produce several generations per year.

Narrow sense heritability
The fraction of phenotypic variance of a trait that can be attributed to additive genetic variance – the variation attributed to the additive effects of genes – that is inherited from parents to offspring. Estimates of narrow sense heritability are used to predict responses to selection over time frames of a few generations.

Ramping assay
Used in assessments of thermal tolerance; individuals are exposed to gradually increasing temperatures until all individuals have succumbed to the thermal stress.

Static assay
Used in assessments of thermal tolerance; individuals are exposed to a set temperature until all individuals have succumbed to the thermal stress.

Soft limits
Population processes such as gene flow and local adaptation that may limit evolutionary adaptation.

Upper knockdown thermal limits
The upper temperature at which an individual can maintain the ability to self-right/move in response to stimuli. Can be assessed using static and ramping assays.

1976; Bridle and Hoffmann, 2022; Hoffmann and Blows, 1994) (Fig. 1). Soft limits (see Glossary) occur when population processes restrict adaptation. For instance, gene flow from central areas of a species range to marginal populations may result in a constant flow of maladapted genes into the latter, effectively preventing the accumulation of genetic variants required for populations to adapt to conditions beyond their current margin (reviewed in Bridle and Hoffmann, 2022). Soft limits can also occur when alleles adapted to average climatic conditions are mostly selected, whereas alleles with a high fitness under extreme climatic conditions are exceedingly rare, and are therefore at a very low frequency, particularly if such alleles have low fitness under average conditions (King and Masel, 2007). Such soft limits are dynamic, resulting from an interplay between the ongoing processes of local adaptation, demography and gene flow along with population marginality. They can (potentially) be overcome by changes to the way alleles are selected and spread across environments such as a reduction in gene flow between central and marginal areas of a species' distribution.

In contrast, hard limits (see Glossary) are characterized by the rarity of the genetic variants required for adaptation; they arise when there is a lack of adaptive genetic variation in key traits across a species range, limiting the ability of the species to adapt to new conditions (Bridle and Hoffmann, 2022; Kellermann et al., 2009). From a classic quantitative genetics perspective, this may be reflected by a very low narrow sense heritability (see Glossary) of the trait under selection (as outlined in Blows and Hoffmann, 2005). Extending to a multivariate perspective, adaptation is limited by available genetic variation across multiple traits that aligns with the direction of selection (McGuigan et al., 2008; Walsh and Blows, 2009). A quantitative genetics perspective on hard limits, while a statistical construct, is nonetheless important because it links genetic variance in traits to selection and evolutionary shifts.
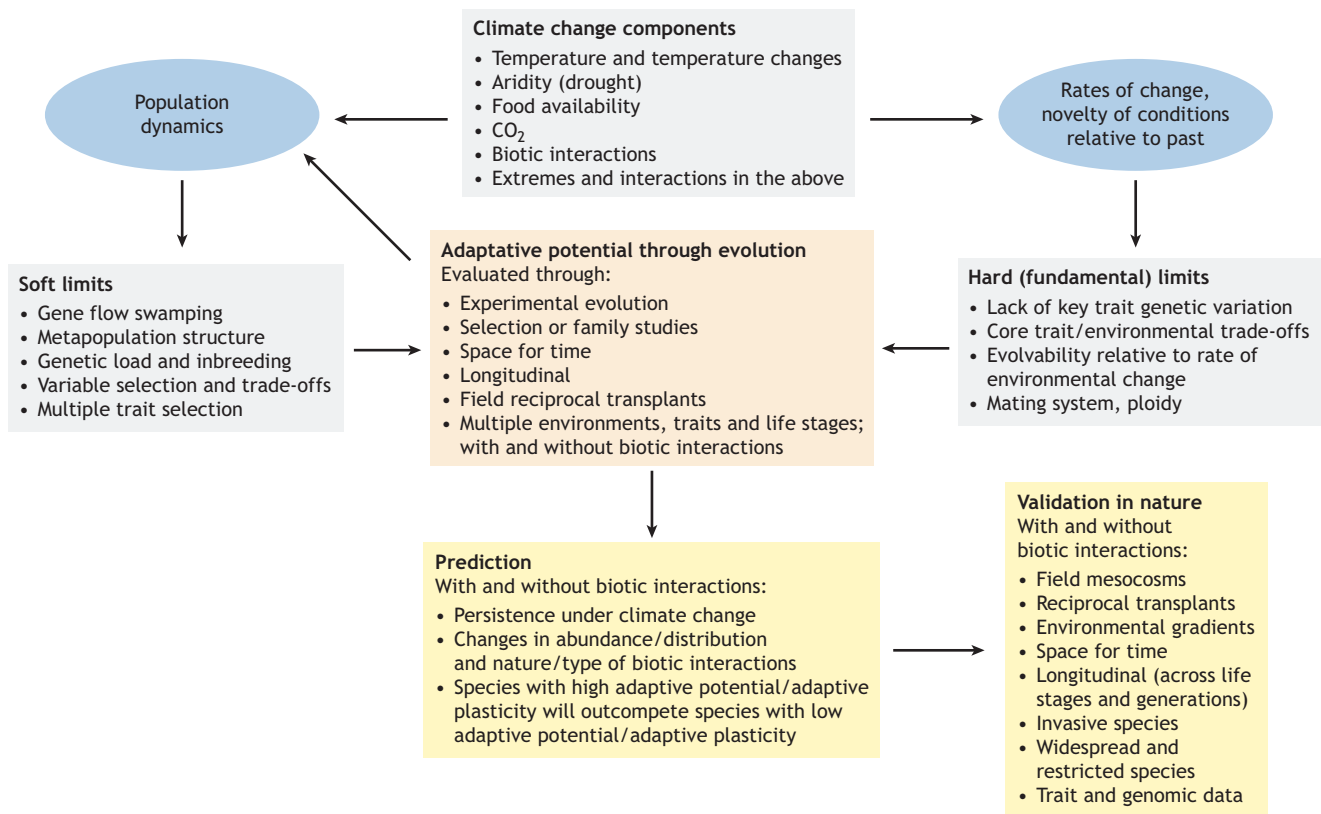
Fig. 1. Conceptual framework for assessing adaptive potential and evolutionary adaptation in invertebrates under climate change. Climate change may directly affect adaptation by imposing selection on traits. But stressors arising from climate change (top box) include biotic as well as abiotic components, which interact to affect organisms and influence population dynamics. Consequently, it is hard to evaluate evolutionary potential (central box), which may be high when there are soft limits (left box) but low when there are hard limits (right box) to adaptation. Predictions of evolutionary adaptation (bottom centre box) need to be verified by comparative studies and field observations (bottom right box), which are still rare in the literature.

Hard limits reflect an absence of organisms with genotypes that can drive a selection response, as reflected by low evolvability or genetic variance (e.g. Hoffmann et al., 2003a; Kellermann et al., 2006), whereas with soft limits, traits can still be genetically variable. A hard limit may prevent traits evolving in a particular direction because of a physiological constraint, as in the evolution of climate sensitivity in multicellular marine organisms restricted by hypoxia and heat (Storch et al., 2014). Overcoming this limit may require substantial genomic changes such as gene duplication or the introgression (see Glossary) of foreign DNA following hybridization. For example, adaptation to arid environments by cactophilic (cactus-associated) *Drosophila* species involved high rates of gene gain (via gene duplications) involving hundreds of genes (Rane et al., 2019), while in *Anopheles* mosquitoes there has been introgression of inversions associated with climate change following species hybridization (Cheng et al., 2018).

Hard limits may also develop over evolutionary time as a consequence of loss of function (LOF) of genes no longer exposed to selection, resulting in the accumulation of nonsense mutations and DNA decay (e. g. Daane et al., 2020; Li et al., 2022). DNA decay in key genes required for adaptation may be difficult to reverse, and it may affect adaptive responses to future environmental change (Daane et al., 2020). The importance of LOF may be particularly strong in drosophilids with a high rate of gene turn over (Hahn et al., 2007) and evidence for multiple genetic changes during radiations (Li et al., 2022). *Drosophila* that are climate specialists (restricted to a narrow range of climates, such as species only found in the wet tropics) tend to have a high rate of DNA decay

overall compared with widespread species, although the specialist species also have overall lower levels of nucleotide variation, which could influence heritable variation (Li et al., 2022).

In contrast, soft limits can be broken when patterns of gene flow or selection are altered, although there are surprisingly few examples of gene flow limiting invertebrate adaptation. Bush crickets from geographically and genetically isolated populations tend to increase in body size with latitude, whereas this association is weak in continuous populations, suggesting that gene flow prevents local adaptation (Cassel-Lundhagen et al., 2011). In the cricket *Allonemobius socius*, asymmetric gene flow from multivoltine populations probably limits the evolution of diapause egg production in marginal higher latitude populations where only univoltine populations persist (Fedorka et al., 2012). A challenge in establishing soft limits is that they require not only estimates of gene flow but also the identification of maladapted genotypes. One issue is that gene flow can be hard to differentiate from environmental factors which impact both selection and gene flow (Bridle and Hoffmann, 2022).

Experimental assessments of evolutionary climate change adaptation

The many experimental designs for assessing evolutionary climate change adaptation (ECCA) vary enormously in terms of the nature of the climate change considered, the extent to which they distinguish whether selection is acting directly on traits or indirectly by tracking populations in shifting environments, and whether designs consider temporal variation in selection. A

straightforward approach to assessing ECCA is to apply a static environmental condition or set of conditions expected to match a component of the future environment, and to expose populations to them to see if any initial decrease in fitness is countered by gradual evolution. More complicated designs include dynamic experimental conditions (Schou et al., 2014; van Heerwaarden and Sgrò, 2021), biotic interactions (Hamann et al., 2021; O'Brien et al., 2022), combinations of stressors (Schneider et al., 2020) or intermittent selection under extremes (Griffin et al., 2017; Michalak et al., 2019). They can also include field-based reciprocal transplant experiments (Buckley and Bridle, 2014; O'Brien et al., 2022) or longer-term observational studies combined with experimental manipulation (van Asch et al., 2013).

The key considerations in these experimental designs come from the factors that directly impinge on the nature of any selection response (or measure of genetic variation). These can influence the outcome of selection, particularly when trade-offs are involved.

Population background
Genetic backgrounds that differ between starting populations may lead to the heterogeneous direct and correlated phenotypic responses to selection that are common across experimental evolution studies (Harshman and Hoffmann, 2000; Kawecki et al., 2012; Michalak et al., 2019). In addition, genetic redundancy (see Glossary) will also contribute to differential genomic responses when using different starting populations or even when measuring genomic changes in replicate lines derived from the same starting population (Barghi et al., 2019; Griffin et al., 2017). Therefore, when different populations are selected for similar traits related to climate adaptation, they may end up at different phenotypic endpoints, or at the same endpoint reached by a different set of genomic changes.

The extent to which populations are adapted to culture conditions (domesticated) can bias the nature and extent of any evolved response to selection (Harshman and Hoffmann, 2000; Kawecki et al., 2012). Any response to selection in domesticated populations adapted to a resource-rich environment might not reflect responses in nutrient-poor and variable natural environments. This is particularly likely if the response to selection involves the accumulation of resources, as can be the case for traits such as desiccation or starvation tolerance. In addition, laboratory adaptation may inadvertently impose selection on traits such as early fertility, which then trade off with stress tolerance traits (Hoffmann et al., 2001). Although the use of laboratory populations introduces problems, there are also issues in using populations not previously exposed to laboratory conditions. These include the introduction of uncontrolled parental effects, which can influence short-term evolutionary responses (Hoffmann and Sgrò, 2018; Johnstone et al., 2017; Kawecki et al., 2012; Schiffer et al., 2013). Some 'evolve and re-sequence' studies (where lines are altered phenotypically in response to selection and then sequenced to test for DNA-level changes to understand the genomic basis of evolved shifts; see Glossary) illustrate how the genomic signals of laboratory adaptation can be distinguished from evolutionary shifts in response to selection (Burny et al., 2022; Griffin et al., 2017) but this separation is difficult at the phenotypic level.

Seasonal variation in the type and strength of selection may also affect the genetic background of starting populations and influence selection responses. Even in the tropics, seasonal changes in precipitation combined with changes in temperature can drive marked genetic variation (e.g. Singh et al., 2020). Seasonally varying clinal patterns for cold tolerance and development time in central versus marginal *Drosophila serrata* populations (Magiafoglou et al., 2002) and allele-frequency variation in *Drosophila melanogaster* (Bergland et al., 2014) highlight the impact of seasonal genetic effects at both the phenotypic and allelic levels. Very rapid genome-wide shifts in allele frequencies and adaptive tracking (see Glossary) have been documented in response to seasonal variation in *Drosophila* field cages (see Glossary; Rudman et al., 2022).

The evolutionary history of a population used to initiate experimental studies can also affect the nature of evolutionary responses under experimental evolution. This includes the specific genetic changes underpinning adaptive shifts, the likelihood of adaptation and the extent to which laboratory-based studies reflect the type of genetic changes needed for adaptation in nature (Kawecki et al., 2012). For example, laboratory-based selection could involve alleles with strong negative pleiotropic effects more often than would occur in nature, overestimating the importance of evolutionary trade-offs in adaptation (Kawecki et al., 2012). Starting populations may also vary in starting frequencies of segregating alleles that vary in effect size, affecting conclusions about the nature of genetic changes underpinning adaptation (Otte et al., 2021). Finally, if the populations assessed have a history of intense selection, or low population sizes, adaptive potential may appear low even when there are no hard limits.

Population size before and during selection
Population size directly affects the rate and extent of evolutionary adaptation (Harshman and Hoffmann, 2000; Kawecki et al., 2012; Langmuller et al., 2021), and the genetic basis of any adaptive shifts. For example, extremely large population sizes (potentially thousands to tens of thousands) will be necessary when rare, low-frequency alleles underpin evolutionary adaptation (Brennan et al., 2019). The ability of evolve and re-sequence experiments to resolve the genomic basis of adaptation may also require population sizes upwards of 5000 individuals (Langmuller et al., 2021). However, even when relatively large populations are used, heterogeneous genomic responses may still be observed as a result of genetic redundancy (Barghi et al., 2019; Griffin et al., 2017).

The way a base population is constructed will influence the amount of standing genetic variation segregating in a starting population (Phillips et al., 2021) and the extent to which the genomic basis of adaptation can be finely mapped (Langmuller et al., 2021). For instance, sets of inbred lines (e.g. the *Drosophila* genetic reference panel, DGRP) and 'synthetic recombinant' populations, which are generated either by pairwise crossing of isogenic strains or by mixing strains in equal proportions (e.g. the *Drosophila* synthetic population resource, DSPR), in *Drosophila*, but also extended to yeasts (Phillips et al., 2021), have become popular to investigate the genomic basis of adaptation in complex traits. Pairwise crossing of isogenic strains results in a higher level of genetic variation in the starting population than mixing many parental strains, but the latter increases genetic diversity. These are useful approaches for investigating the genomic basis of selection responses but minimizing linkage disequilibrium (the extent to which genetic markers in proximity to each other on the same chromosome are coinherited) in the starting population may be important in linking results to selection responses in natural populations (Phillips et al., 2020). The length of time allowed for recombination to break up linkage disequilibrium after lines have been mixed/crossed to generate starting populations (and before selection commences) will be important, especially if inversions segregate within the recombinant lines.

Length of experiment

Experiments using dynamic assay conditions to test whether adaptation extends the time to extinction for different species (e.g. van Heerwaarden and Sgrò, 2021) may of necessity only run for a few generations; likewise, tests for adaptive shifts in single traits often run for only a few generations (Harshman and Hoffmann, 2000; Kawecki et al., 2012), particularly if extremely large populations are exposed to very strong selection (Brennan et al., 2019). Short-term experiments can provide strong evidence of the capacity to adapt but may not reveal the contribution of rare alleles and novel genomic changes to adaptation in the longer term [see 'Population (soft) versus fundamental (hard) limits', above].

Length issues have come into focus with evolve and re-sequence experiments. Although some studies use 15–20 generations of selection (e.g. Griffin et al., 2017; Langmuller and Schlotterer, 2020), others have used far more (e.g. hundreds; Burke et al., 2010). When the aim of studies is to extrapolate to population and species differences and investigate factors such as LOF, long time frames will be needed. Short-term experiments may be insufficient to distinguish whether the absence of a response is due to hard limits or to other issues such as linkage disequilibrium (see above). The number of sampling times also matters in evolve and re-sequence experiments, where multiple sampling periods help resolve the dynamic nature of the evolutionary process (Phillips et al., 2020).

Focus traits

Ideally, measures should target ecologically relevant traits, such as climatic stress tolerance, that capture vulnerability and resilience to climate change. This is challenging because of the many different traits contributing to climate change adaptation across a life cycle (Bowler and Terblanche, 2008; Kingsolver et al., 2011), and the relative sensitivity of traits to stresses across an invertebrate's life-cycle stages which, in turn, needs to be related to the microenvironment experienced at that stage (Fig. 2). For example, estimates of upper knockdown thermal limits in adults (see Glossary; Box 1) can differ widely, depending on the rate of temperature increase/intensity and duration of the heat stress (Jørgensen et al., 2021; Rezende et al., 2014; Terblanche et al., 2007). Furthermore, studies that have directly compared upper lethal thermal limits (see Glossary) across life stages in *Drosophila*, butterflies, leaf miners and others have often found upper lethal limits to be lower at earlier life stages, so that studies focusing solely on adults might underestimate vulnerability (Klockmann and Fischer, 2017; Moghadam et al., 2019; Pandori and Sorte, 2019; Pincebourde and Casas, 2015) (Fig. 3). These issues also apply to other stresses (e.g. cold tolerance Jensen et al., 2007).

Life stages may experience different environmental conditions as a result of variation in mobility and capacity to avoid stresses through behavioural regulation (Huey et al., 2012; Levy et al., 2015; Sunday et al., 2014), or because they occupy different habitats/microhabitats or encounter different seasons (Bowler and Terblanche, 2008; Kingsolver and Buckley, 2020; Kingsolver et al., 2011). Consequently, estimates of tolerance and adaptive potential in the laboratory need to be linked to relevant conditions in nature. For example, in the leaf miner *Phyllonorycter blancardella*, upper thermal limits were higher at later life stages, yet vulnerability to climate change was similar across life stages because temperatures were also higher in the microclimates experienced by later life stages (Pincebourde and Casas, 2015). In the butterfly *Boloria eunomia*, increases in temperature increased egg, pre-diapause larval and pupal survival and female fecundity, but the

survival of overwintering larvae declined, which reduced population viability under climate change scenarios (Radchuk et al., 2013).

Although lethal limits can set upper thermal limits for development and survival across the life cycle, other important fitness traits – such as reproduction – may be more sensitive to temperature and underpin vulnerability (Jørgensen et al., 2022; van Heerwaarden and Sgrò, 2021; Walsh et al., 2019) (Fig. 3, Box 1). For instance, studies on *Drosophila*, flour beetles, wasps, moths and flesh flies have found that male fertility may be negatively affected by high temperatures well below their upper lethal activity limit or developmental thermal limits (Nguyen et al., 2013; Parratt et al., 2021; Rinehart et al., 2000; van Heerwaarden and Sgrò, 2021; Zhang et al., 2015), suggesting that knockdown or lethal temperatures estimated in adults, and developmental limits across the life cycle, may underestimate the impacts of high temperatures. Although fertility loss due to high temperature often occurs at lower temperatures in males than in females (Rinehart et al., 2000; Sales et al., 2018; van Heerwaarden and Sgrò, 2021; Zwoinska et al., 2020), females can also be affected by heat damage to stored sperm (McAfee et al., 2020; Sales et al., 2018; Walsh et al., 2022).

Understanding impacts across the life cycle is further complicated by environmental effects at earlier life stages carrying over to other life stages (Kingsolver et al., 2011; Klockmann and Fischer, 2017; Rebolledo et al., 2021; Zhao et al., 2019). Exposure to warmer or stressful temperatures during development may activate hardening (see Glossary) or beneficial acclimation responses, which might increase tolerance in subsequent life stages (Gunderson and Stillman, 2015; Pottier et al., 2022; Sgrò et al., 2016). Conversely, the effects of high temperatures during development may carry over to later life stages through the accumulation of damage or changes in growth/developmental rates and resource acquisition (Boggs and Freeman, 2005; Schluter et al., 1991). This may reduce body size and condition, decreasing thermal tolerance, reproductive success and competitiveness (Kingsolver and Huey, 2008). As noted above, stressful temperatures during development may be particularly detrimental to male fertility (David et al., 2005; van Heerwaarden and Sgrò, 2021; Zwoinska et al., 2020), with fertility effects differing depending on which life stage is stressed (Parratt et al., 2021; Rinehart et al., 2000). Effects on adults may also carry over to early life stages of the next generation (Schiffer et al., 2013; Sikkink et al., 2014; Uller et al., 2013; Waite and Sorte, 2022), possibly as a result of transgenerational effects (Donelson et al., 2012; McGuigan et al., 2021) or trade-offs between parental condition and investment in offspring (Marshall and Uller, 2007). For example, in the nematode *Caenorhabditis remanei*, heat shock tolerance was lower in larvae when parents experienced a heat stress (Sikkink et al., 2014). Life stages might also differ in their capacity to tolerate thermal stress through plasticity (hardening, acclimation within and across life stages). For example, in *D. melanogaster*, hardened sessile pupae survived temperatures that killed 80% of unhardened adults despite the former having a lower unhardened lethal thermal limit (Fig. 3; and see Moghadam et al., 2019). Evolutionary changes in strategies such as reproductive diapause/quiescence that allow an individual to avoid stressful environmental conditions (Masaki, 1980), or changes in diurnal activity patterns (Gotcha et al., 2021; Moghadam et al., 2019) are also important for allowing a species to adapt to climate change.

One option to identify traits that are the targets of selection under future climate change is to look for traits that have evolved across climatic gradients (Hoffmann and Sgrò, 2011; Verheyen et al., 2019), the evolutionary equivalent of 'space for time' (changes in
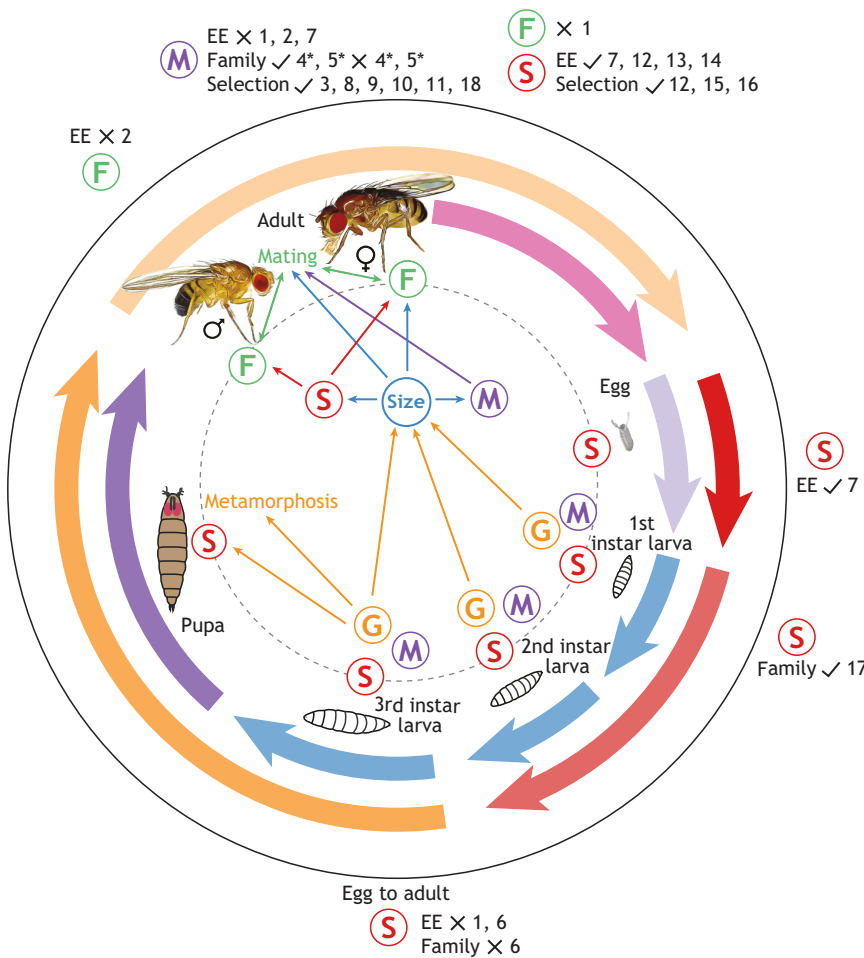
Fig. 2. Complexity of the life cycle when assessing adaptive responses, as illustrated for a fruit-breeding *Drosophila* species such as *D. melanogaster*. Different life stages can vary in size, morphology, timing of initiation and termination, and mobility. These fitness components/traits (circles) can be selected and may be used to assess vulnerability/ adaptive response to climate change. S, survival; G, growth rate or time; M, movement (activity, flight, running speed); Size, body size (and pigmentation); F, fertility (male fertility traits may include sperm production, sperm survival, fertility recovery; female fertility traits may include fecundity, reproductive diapause, sperm storage). Exposure to thermal stress in earlier life stages might impact tolerance, size and reproduction in subsequent stages through plasticity, accelerated growth rate and trade-offs in resource allocation. Examples of studies examining adaptive potential to heat stress across different life stages are indicated outside the black circle. EE, experimental evolution; Family, family studies (e.g. parent–offspring or sibling studies); Selection, direct or indirect (family) selection experiments. Crosses and ticks indicate whether a selection/evolutionary response or significant heritability was detected for increased heat tolerance. Numbers refer to references below. *Estimates of heritability depended on whether heat knock down was measured using static or dynamic heat stress. [1]Schou et al. (2014); [2]van Heerwaarden and Sgrò (2021); [3]Gilchrist and Huey (1999); [4]Mitchell and Hoffmann (2010); [5]Blackburn et al. (2014); [6]Kristensen et al. (2015); [7]Gilchrist et al. (1997); [8]Krebs and Thompson (2006); [9]McColl et al. (1996); [10]Bubli et al. (1998); [11]Huey et al. (1992); [12]Stephanou and Alahiotis (1983); [13]Cavicchi et al. (1995); [14]Huey et al. (1991); [15]Morrison and Milkman (1978); [16]Bubliy and Loeschcke (2005); [17]Krebs and Feder (1997); [18]Hangartner and Hoffmann (2016).

climate across space equivalent to those predicted in time). Heritable clines in traits such as size, pigmentation, reproduction, growth/development rate, flight performance, heat, desiccation and cold tolerance along latitudinal, altitudinal or urbanization gradients all co-vary with environmental variables (Clusella-Trullas and Nielsen, 2020; Hoffmann et al., 2003b; Verheyen et al., 2019). However, these patterns may not necessarily indicate traits that are important for vulnerability and future persistence. For example, body size clines have been detected for many species, and size is likely to be under climatic selection (Chown and Gaston, 2010; James et al., 1995; van Heerwaarden and Sgrò, 2011), but body size may not affect persistence under climate change.

Thermal limits in male fertility may be a better predictor of climate change vulnerability than those affecting survival and development, as fertility thermal limits more closely associate with environmental variables across *Drosophila* species than CT_{max} (the upper critical thermal limit for knockdown or survival) and other fitness traits (Parratt et al., 2021; van Heerwaarden and Sgrò, 2021). Upper fertility thermal limits in males developing at fluctuating stressful temperatures were also better predictors of extinction temperatures (see Glossary) under simulated warming (van Heerwaarden and Sgrò, 2021). Mechanistic models may help pinpoint the types of traits and environmental conditions that should be assessed (Maino et al., 2016), and transplant experiments along an ecological gradient can help link variation in ecologically important traits to field fitness (O'Brien et al., 2022). However, a challenge in all these comparisons is understanding adaptation to rare extreme events (Elderkin et al., 2004).

Environmental variability

Environmental tolerance varies depending on environmental conditions, affecting estimates of vulnerability. Differences in tolerance under fluctuating temperatures can occur because the relationship between performance/tolerance and temperature is generally non-linear (Jensen's inequality; see Glossary), which causes thermal tolerance/performance to increase or decrease under fluctuating environments, depending on the average temperature and the amplitude, duration and symmetry of fluctuations (Colinet et al., 2015; Ruel and Ayres, 1999). Differences in tolerance/ performance under fluctuating temperatures can be integrated into thermal death time (TDT) (Jørgensen et al., 2021) and thermal performance curve (TPC) models (Colinet et al., 2015). These models apply to performance/tolerance across one set of temporal scales (i.e. permissible or acute temperature ranges) (Jørgensen et al., 2021), which does not account for the accumulation of damage when temperatures fluctuate outside these ranges and the accumulation of damage across generations. Cumulative measures may not be able to cover changes in both average and extreme temperature events (Colinet et al., 2015; Jørgensen et al., 2021), and also may not capture the potential for damage to be repaired when species return to permissible temperatures (Bowler and Kashmeery, 1979; Jørgensen et al., 2021; Ørsted et al., 2022) – an important issue under asymmetrical patterns of diurnal temperature variation, with detrimental effects of night-time warming reducing repair potential (Speights et al., 2017; Zhao et al., 2014). Recovery rate itself may be species specific and temperature dependent (Ørsted et al., 2022). Plasticity (see 'Focus traits', above) also affects

Box 1. Estimating thermal limits

Estimates of thermal limits are widely utilized to compare thermal tolerance but studies vary in what terminology they use (e.g. critical thermal maximum, upper lethal limit, fertility thermal limit, thermal fertility limit, upper lethal limit, LT_{50}), the traits or endpoints assessed (e.g. movement, death, onset of spasms, fitness, fertility) and the timing, duration and intensity of thermal stress. Critical thermal maximum (CT_{max}) can be estimated from thermal performance curves (TPCs), which measure how performance changes across a temperature range (A). Here, we used a dataset from *Drosophila hydei* (Overgaard et al., 2014) for calculating critical thermal minimum (CT_{min}), thermal optima (T_{opt}) and CT_{max} using TPCs assessing fitness across multiple long-term temperature treatments. Performance could be fitness across a life cycle but is often measured using single performance traits (e.g. developmental viability/ time, running speed, metabolism, fecundity). CT_{max} can also be estimated over short time scales (minutes or hours) by measuring knockdown temperatures of organisms (usually adults) exposed to acute ramping temperatures (which can differ in duration, ramping rate and starting temperature) or thermal death time curves (TDT) (B). TDT curves describe thermal tolerance using the slope of the relationship between assay temperature and $\log_{10}$ coma time, using assessments of knockdown time at various acute static temperatures. This slope can be used to calculate the thermal sensitivity coefficient (Z) (-1/slope) and static CT_{max} (sCT_{max}), the temperature causing knockdown after set durations. The example here for *Drosophila buzzatii* (Jørgensen et al., 2019) shows the relationship between temperature and $\log_{10}$ knockdown time in the stressful temperature range, and sCT_{max} for 1 h (purple dotted lines). CT_{max} estimated from dynamic assays (open circle, square and triangle; Jørgensen et al., 2019) using different ramping rates is also shown. Upper lethal thermal limits (LTLs), sometimes called upper thermal limits (UTLs), can be estimated at adult and pre-adult life stages, and assessed using different temperature treatments and different lethal thresholds (e.g. LT_{80}, the temperature that kills 80% of individuals) (C). Recently, studies have been estimating upper fertility thermal limits (FTLs) in males after developing at high temperatures, or acute heat shocks (thermal fertility limits, TFLs) (van Heerwaarden and Sgrò, 2021; Parratt et al., 2021). The example here for *D. buzzatii* represents a dataset (van Heerwaarden and Sgrò, 2021) for calculating male fertility (yellow) and egg-to-adult survival (blue) upper thermal limits ($FTL_{50/80}$ and $VTL_{50/80}$, respectively; dotted horizontal lines) using fluctuating temperatures during development (dashed lines), and a dataset (Parratt et al., 2021) assessing male fertility (green) and survival (red) upper thermal limits ($TFL_{50/80}$ and $LTL_{50/80}$, respectively; dotted horizontal lines) using short-term acute temperatures on adults (solid lines).
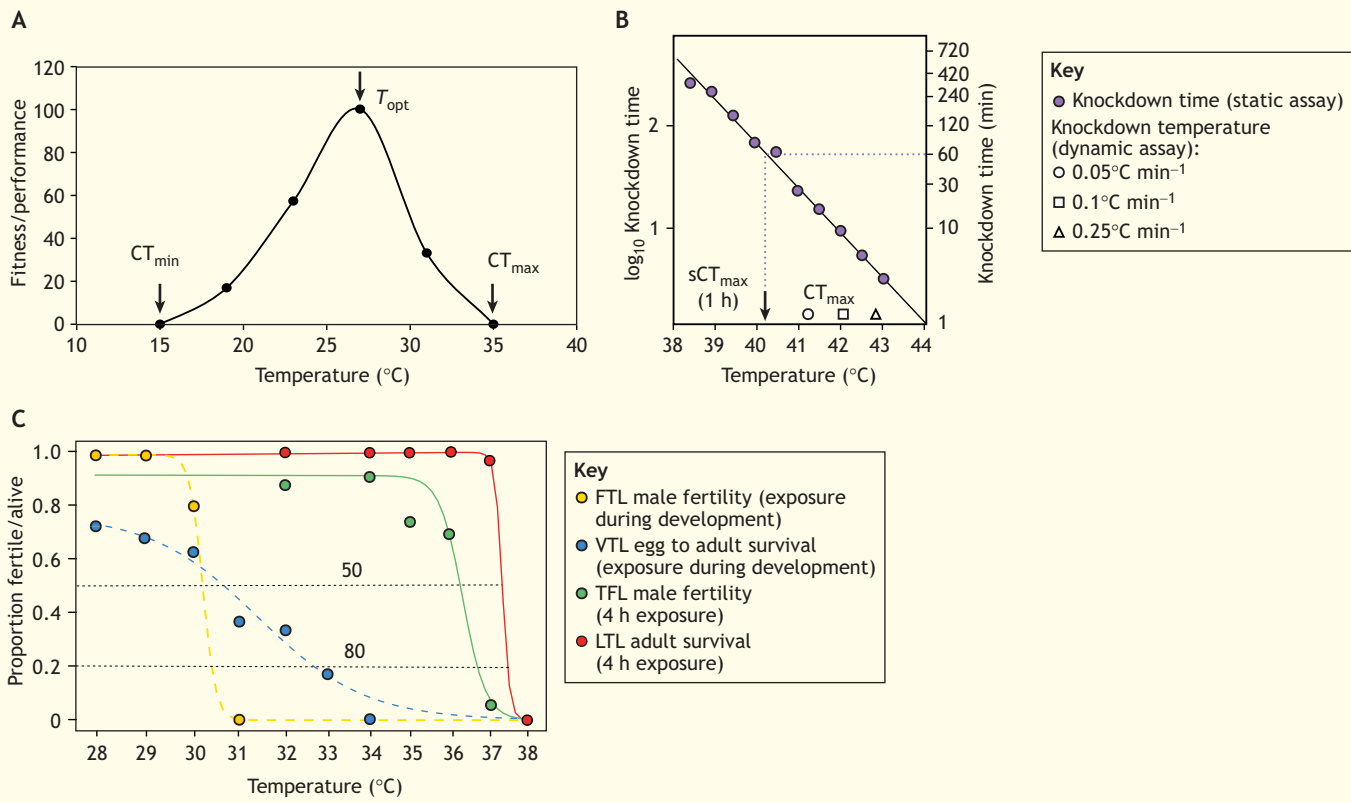
tolerance and damage repair, and little is known about repair and plasticity for organismal traits such as the thermal susceptibility of fertility (Parratt et al., 2021).

Environmental variability will have complex effects on adaptive capacity not only through direct effects on traits but also because it affects selection and the expression of additive genetic variance and heritability (Falconer and Mackay, 1996; Hoffmann and Merila, 1999; Hoffmann and Parsons, 1991). Laboratory estimates of heritability are often only valid for the environment in which they are measured. For example, in two rainforest *Drosophila* species, additive genetic variation and heritability for heat tolerance were higher in flies exposed to temperature fluctuations during development that mimicked future summer conditions compared with those mimicking winter

temperature fluctuations or constant 25°C (van Heerwaarden et al., 2016). Direct comparisons of heritability in constant and fluctuating environments have produced mixed results as in the case of egg to adult viability in *D. melanogaster* (Ketola et al., 2012; Kristensen et al., 2015). Impacts on selection depend on whether lower heritability coincides with conditions when selection is strong or weak (Husby et al., 2011; Wilson et al., 2006).

Additionally, environmental variability will affect evolution by influencing selection across life stages and generations. Evolutionary responses may be hindered if adaptation to an environmental condition at one time point is maladaptive to different conditions later (Kingsolver and Buckley, 2015). However, fluctuating conditions can also help increase evolutionary responses, by overcoming potential
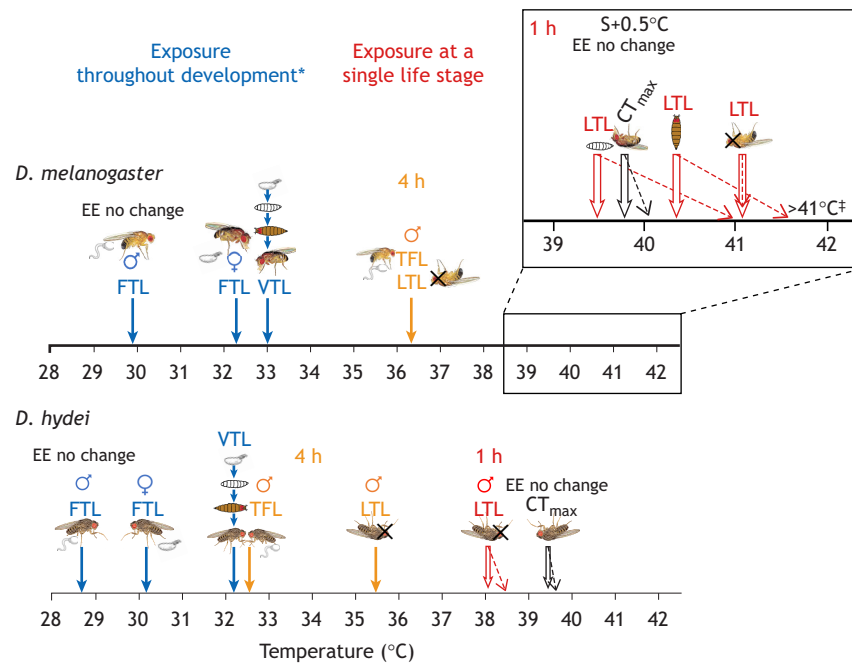
Fig. 3. Upper thermal limits in two cosmopolitan *Drosophila* species – *D. melanogaster* and *D. hydei* – can differ depending on the trait(s) used, life stage(s) exposed to thermal stress, life stage(s) assessed, duration of the thermal stress and whether plastic or adaptive responses are considered. The upper thermal limit for male fertility (fertility thermal limit, FTL: 80% sterility) under fluctuating temperatures during development for both species (blue arrows) is lower than the female fertility thermal limit and upper limit for successful development from egg to adult (viability thermal limit, VTL: 80% unsuccessful development) (van Heerwaarden and Sgrò, 2021). In *D. melanogaster*, the 80% male thermal fertility limit (TFL) after a 4 h heat shock is the same as the lethal thermal limit (LTL: 80% mortality), whereas in *D. hydei*, the male 80% TFL after a 4 h heat shock is much lower than the 80% LTL (orange arrows) (Parratt et al., 2021). In *D. melanogaster*, the 80% upper lethal limit (LTL) after a 1 h heat shock is lower for larvae and pupae than for adults (open red arrows); following hardening (dashed red arrows), pupae have the highest LTL (Moghadam et al., 2019). CT_{max} (upper knock-down temperature, here measured at a ramping rate of 0.1°C increase per minute) for both species is also shown (open black arrows) along with the effects of hardening (dashed black arrows) (Kellermann and Sgrò, 2018). The 1-h 80% lethal limit for adult *D. hydei* (open red arrow) and plastic response to a 1-h hardening treatment at 37°C (dashed red arrow) are also shown (S. Macdonald, X. Gu and B. van Heerwaarden, unpublished). Evolutionary responses in male FTLs and CT_{max} under experimental evolution (EE) to warming (0.1°C per generation) were not apparent in both species (van Heerwaarden and Sgrò, 2021). A 0.5°C increase in CT_{max} was observed in *D. melanogaster* after 10 generations of selection (S) for increased heat knock down (Hangartner and Hoffmann, 2016). ‡Temperatures above this were not tested. *Fluctuating temperatures were used.

negative carry-over effects, or by allowing population size to recover. For example, in the marine diatom *Thalassiosira pseudonana*, long-term evolution under a temperature regime that fluctuated every three to four generations between constant 22 and 32°C resulted in faster adaptation to warming compared with adaptation to constant 32°C and was similar to responses to more moderate warming (constant 26°C) (Schaum et al., 2018).

Is there an optimal way to measure ECCA?
Given the challenges discussed above, evolutionary adaptive potential may need to be assessed on a case-by-case basis to reflect the (future) environment experienced by an organism. But then what is the best approach? As highlighted in earlier reviews (e.g. Hoffmann et al., 2003b, 2013), each approach has its limitations. Family studies (see Glossary) may lack the power to accurately estimate heritability, especially in physiological and fitness traits with measurement error/noise. Selection experiments may be more powerful in detecting low genetic variation (Hangartner and Hoffmann, 2016), but are also limited by design, particularly if carry-over effects across generations limit responses (Anderson et al., 2005). Both family and selection experiments struggle to capture life-stage complexities. Experimental evolution using mixed generations in large cages, across multiple life stages may be 'more representative' of nature (Cavicchi et al., 1995; Esperk et al., 2016; van Heerwaarden and Sgrò, 2021). However, this approach

can inadvertently select for other traits; for instance, if there is too much variation in larval density then life-history traits favoured under high density conditions such as rapid development can be favoured (Bubli et al., 1998; Santos et al., 1997) even though these traits are unconnected to ECCA. Furthermore, as discussed above, differences in sensitivity, microclimates and seasonal environments across life stages remain difficult to capture in the laboratory, regardless of the approach used (Kingsolver et al., 2011). Capturing phenological effects in the laboratory is also challenging when conditions triggering phenological events are often poorly defined, although clinal patterns can provide a starting point, as in the genetic analysis of diapause in the butterfly *Pararge aegeria* (Pruisscher et al., 2018).

In search of patterns
Despite the challenges highlighted above, are generalizations about ECCA emerging from evolutionary studies on *Drosophila* and other invertebrates? For instance, how often are hard selection limits encountered for increased thermal resistance and to what extent are limits set by trade-offs among traits?

Heritable variation seems low for some classes of traits, but also varies across species and different trait measures
Data from family, selection and evolution experiments suggest that although heritable variation for heat tolerance can often be detected (Diamond, 2017) and may even be high in some species (Ma et al.,

2014), heritability is often very low (Blackburn et al., 2014; Elderkin et al., 2004; Mitchell and Hoffmann, 2010). Selection responses are also often small and plateau after a few generations (Gilchrist and Huey, 1999; Hangartner and Hoffmann, 2016; Kelly et al., 2012), suggesting a hard upper limit for heat tolerance generally (Kellermann et al., 2012). However, a 2 year experimental evolution experiment in outdoor mesocosms on the water flea *Daphnia magna* found a 3.6°C genetic increase in heat tolerance in response to +4°C selection treatment warming, and the resurrection of dormant eggs from sediment layers also indicated the evolution of higher heat tolerance (Geerts et al., 2015).

For male fertility at high temperatures, the question of whether there is much heritable variation in invertebrates remains unclear. Broad sense heritability (see Glossary) for male fertility at high developmental temperatures in *D. melanogaster* lines from the DGRP was very low (Zwoinska et al., 2020). An experimental evolution study found no change in male fertility in multiple *Drosophila* species under rising temperatures (van Heerwaarden and Sgrò, 2021), suggesting little capacity to increase fertility under sterilizing developmental conditions. However, there may be some intraspecific genetic variation in the sterilizing temperature and recovery period (David et al., 2005).

Desiccation tolerance also shows a variable response to selection across species even when these are related. It responds rapidly to selection in widespread *Drosophila* species but not others (Hoffmann et al., 2003a), and heritability of tolerance to severe desiccation stress appears to be limited in tropical desiccation-sensitive species (Kellermann et al., 2009). A similar pattern is evident for species sensitive to cold stress (Kellermann et al., 2009), suggesting that low heritability in sensitive *Drosophila* species may represent a hard limit, as multiple populations extending across their distribution have extremely low heritability for these traits (Kellermann et al., 2006), which is unlikely to be driven by population processes given that neutral marker diversity/additive genetic variation for morphological traits in the same species is high (van Heerwaarden et al., 2009).

However, estimates of heritability can differ depending on how aridity tolerance and thermal stress tolerance are measured (Blackburn et al., 2014; Mitchell and Hoffmann, 2010; van Heerwaarden and Sgrò, 2013), highlighting the importance of assessing traits under conditions relevant to current and future conditions. For example, heritability for desiccation tolerance in rainforest species of *Drosophila* depends on whether severe or moderate desiccation stress was used (van Heerwaarden and Sgrò, 2014). Heritability for heat knock-down time (see Glossary) differs depending on whether static assays or ramping assays (see Glossary) are used (Blackburn et al., 2014; Mitchell and Hoffmann, 2010; van Heerwaarden and Sgrò, 2013), suggesting that these traits have a different genetic basis. However, there are shared genetic mechanisms (perhaps involving the expression of heat shock proteins) partly underlying different measures of heat knock-down tolerance (Mesas et al., 2021; Sgrò et al., 2010; van Heerwaarden et al., 2012), and cold tolerance (Anderson et al., 2005; Udaka et al., 2010). Nevertheless, artificial selection to increase knock-down time or recovery under heat stress may fail to increase heat survival after an acute stress (Bubliy and Loeschcke, 2005; Gilchrist et al., 1997; Hoffmann et al., 1997; Udaka et al., 2010).

There is often independence of traits across life-cycle stages but genetic interactions with life-history traits are common

Previous reviews of *Drosophila* data (Hoffmann et al., 2003b) and recent studies (Dierks et al., 2012; Freda et al., 2019) suggest that thermal tolerance across life stages is largely genetically independent. For example, when *Drosophila buzzatii* adults and larvae were independently selected for heat tolerance, responses were only observed in the life stage selected (Loeschcke and Krebs, 1996). Quantitative trait locus (QTL) studies also suggest that thermal tolerance is not genetically correlated across life stages in *D. melanogaster* (Freda et al., 2019).

Trade-offs have classically been considered in the context of life-history evolution, whereby selection on one trait causes others to change as a consequence of genetic correlations (Roff, 1992). This framework considers the way individual organisms allocate resources between two traits (such as survival and reproduction), using Y-shaped or bifurcation models (Cushing and Stefanko, 2021). Various versions of this model consider not only energy flow, such as the energetic trade-offs associated with flight/dispersal (Nespolo et al., 2008), but also the need to balance investment of space resources, such as the space required for flight muscles versus eggs and other components of the reproductive machinery. Climate change adaptation in invertebrates through increased mobility to avoid stressful conditions is therefore expected to trade off with reproduction – as is well established in the sand cricket, *Gryllus firmus* (Roff and Fairbairn, 2007b) – and may contribute to changes in phenotypic traits under recent climate change, as seen in water striders (Harada et al., 2011).

Traits directly linked to climatic stress tolerance may also trade off against each other. For example, starvation tolerance and cold tolerance established from selection experiments and other approaches often have trade-offs (Bubliy and Loeschcke, 2005; Hoffmann et al., 2003b), which may relate to resource allocation to protect cells as opposed to being used as energetic resources. In contrast, tolerance to thermal stresses tends to be weakly correlated with desiccation tolerance in *Drosophila*, although there are specific genes that may have opposing effects on these traits; for instance, knockout of the *hsp23* gene by CRISPR/Cas9 decreases cold tolerance, but increases heat tolerance, in *D. melanogaster* (Gu et al., 2021).

In assessing the impact of trait interactions on climate change adaptation, it is important to consider the nature of selection, as mentioned above. And it is also important to consider the temporal pattern of selection, particularly with climate change impacts often involving rare extreme conditions. Selection might then favour phenotypes under optimal conditions at the expense of those favoured at extremes, highlighting the importance of investigating interactions between stress tolerance traits and those involving biotic interactions, such as predation (Hangartner et al., 2017) or competitive ability (Mauro et al., 2021).

Negative genetic correlations may occur between plastic and non-plastic (basal) responses to climatic stress, which, in turn, could constrain evolutionary responses (Ørsted et al., 2019; van Heerwaarden and Kellermann, 2020). For instance, copepods selected for increased heat tolerance show reduced thermal plasticity (Sasaki and Dam, 2021), whereas *D. melanogaster* lines with increased cold tolerance show decreased cold plasticity under some conditions (Ørsted et al., 2019). Genetic correlations among life-history traits may change generally with environmental conditions (Ørsted et al., 2019; Sgrò and Hoffmann, 2004), and may also change across developmental stages (Freda et al., 2019). This affects our ability to pinpoint and predict responses to climate change that involve components of plasticity as well as genetic changes.

Theory suggests that genetic variances and covariances (G matrix) and, thus, genetic correlations can evolve (Hangartner et al., 2020; Roff and Fairbairn, 2007a), yet empirical evidence is mixed (Hangartner et al., 2020; Ørsted et al., 2019). With stress

tolerance traits typically showing a polygenic basis in *Drosophila* (e.g. desiccation and other stressors; Griffin et al., 2017; Michalak et al., 2019), there are many possible outcomes in terms of trait interactions, and these may need to be evaluated on a case-by-case manner, such as through an evaluation of the variance–covariance matrix across different conditions (Hangartner et al., 2020).

Predictions and future directions

Data on heritable variation can be used in predictive models to understand the potential of evolutionary changes to influence changes in species distributions under climate change. An early invertebrate example involved predicting the future distribution of the mosquito *Aedes aegypti* in northern Australia, where variation in the tolerance of eggs to increasingly dry conditions had a major impact on the ability of this mosquito to persist around a population centre (Kearney et al., 2009). Other modelling applications in invertebrates include predicting the impact of heritable variation on the persistence of threatened *Drosophila* species under high temperature extremes (Bush et al., 2016).

Clearly, a challenge in these applications is to understand whether estimates of heritable variation in such models are realistic and relevant to field conditions. In the case of *Ae. aegypti*, no direct estimates of heritable variation for desiccation tolerance in eggs were available, although there is evidence for genetic divergence of this trait across populations (Faull and Williams, 2015). We have already pointed out the low heritability for heat tolerance traits in many species, and also the lower heat limits likely to be associated with male fertility, which need to be evaluated for genetic variation.

Combining across multiple stages and environments

As we have emphasized above and in Figs 2 and 3, one important aspect in assessing vulnerability to climate change in invertebrates is that the entire life cycle needs to be considered. This is partly because life stages can vary inherently in stress sensitivity and their ability to avoid it through acclimation and behavioural changes (Mitchell et al., 2013), but also because stress exposure at one life stage influences tolerance at a later stage as demonstrated in both aphids (Zhao et al., 2019) and moths (Zhang et al., 2015). In addition, changing environmental conditions can have cumulative effects on tolerance, building up heat stress damage beyond a threshold to exceed a mortality threshold (Jørgensen et al., 2019), and preventing organisms repairing stress damage (Speights et al., 2017). While challenging, future studies should obtain measurements of evolutionary potential under natural/semi-natural conditions that encompass different life stages and relevant environmental conditions. Field-based mesocosms, reciprocal transplants and multi-generation assessments of traits/genomes could help, particularly if carried out in designs where experimental warming or drought conditions are imposed. Collaborations that combine experimental approaches and span countries/continents may be one way of enabling such research programmes, as has been implemented successfully in ecological studies of global change (e.g. The Nutrient Network; Borer et al., 2014). We also emphasize the need for more work on evolutionary adaptation through stress evasion based on traits such as diapause and behavioural avoidance, which often vary clinally in invertebrates including *Drosophila* (Schmidt and Conde, 2006) and water striders (Blanckenhorn and Fairbairn, 1995).

Incorporating biotic interactions

The introduction of biotic factors provides further complexity in evaluating evolutionary changes. They impact the extent to which selection favours local climate adaptation (O'Brien et al., 2022) and have a direct impact by trading off against stress tolerance. Some of the best-known examples of climate change affecting ecosystems involve biotic interactions. For instance, bark beetles have caused enormous tree mortality in the northern hemisphere, both directly and through their interactions with the microbial community that affects the damage (Sallé et al., 2014; Stadelmann et al., 2013). Evolutionary changes in response to biotic resources will often be complex and unpredictable. For instance, in brown argus butterflies, high-dispersal genotypes colonizing new areas available under climate change have unexpectedly become more specialized on their plant hosts (Bridle et al., 2014). While biotic interactions may affect species susceptibility to climate change (Alton and Kellermann, 2023; Hector et al., 2022, 2021) and predictions of range shifts under climate change (Davis et al., 1998), it is not known whether biotic interactions have much direct impact on the expression of adaptive genetic variation and evolutionary potential; this could be tested experimentally.

Testing predictions

Experimental results on evolutionary adaptation in invertebrates have rarely been linked to climate change effects under field situations. Above we have discussed a few examples, such as the evolution of heat tolerance in *Daphnia*. Other examples include: (1) abundance changes in two pest aphids in China where repeated heat waves have favoured the aphid species capable of genetic adaptation and showing cross-generation plasticity (Zhu et al., 2021); (2) *Colias* butterflies that have evolved wing melanization patterns that benefit thermoregulation under warming conditions (Nielsen and Kingsolver, 2020); (3) the classic case of diapause induction evolution in pitcher plant mosquitoes (Bradshaw and Holzapfel, 2008); and (4) changes in dispersal phenotypes in water striders (Harada et al., 2011). Evolutionary changes have also been detected in invasive invertebrate species so that their climate niche has expanded (e.g. Hill et al., 2013), suggesting rapid evolution once populations are no longer constrained by soft selection limits as a result of asymmetric gene flow. Many more studies are needed where predictions from experimental data are matched to the presence (or absence) of evolutionary changes detected under field conditions that include biotic interactions (Fig. 1).

Conclusions

In some ways, we have made a lot of progress in evaluating the evolutionary potential of invertebrates to adapt to climate change, highlighting the presence of genetic variation for many (but not all) traits that are relevant to climate change, demonstrating selection responses in laboratory environments that are expected to simulate some aspects of climate change, and showing past evidence of adaptation to climatic gradients. However, there is complexity in the dependence of adaptive responses on the environmental context that influences both the expression of genetic variation and the nature of selective processes across time. This is particularly the case when extreme and uncommon events are often defining the ecological impact of climate change. We suspect that separation between soft and hard selection limits is a useful framework to assess evolutionary vulnerability and to direct genomic efforts aimed at evaluating climate change adaptation across groups of species. Far more effort is needed to track evolutionary changes (or the lack of them) across related species where it is possible to link laboratory data to field outcomes. Clearly, there is evidence for climate adaptation in invertebrates that have encountered new climate conditions, and with additional studies that extend from the

laboratory to the field level, we hope that more generalities will emerge about predicting vulnerability.

Competing interests

The authors declare no competing or financial interests.

References

Altermatt, F. (2010). Climatic warming increases voltinism in European butterflies and moths. *Proc. R. Soc. B* **277**, 1281-1287. doi:10.1098/rspb.2009.1910

Alton, L. A. and Kellermann, V. (2023). Interspecific interactions alter the metabolic costs of climate warming. *Nat. Clim. Change* **13**, 382. doi:10.1038/s41558-023-01607-6

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. *Genet. Res.* **85**, 15-22. doi:10.1017/S0016672304007281

Antonovics, J. (1976). Nature of limits to natural-selection. *Ann. Mo. Bot. Gard.* **63**, 224-247. doi:10.2307/2395303

Barghi, N., Tobler, R., Nolte, V., Jaksic, A. M., Mallard, F., Otte, K. A., Dolezal, M., Taus, T., Kofler, R. and Schlotterer, C. (2019). Genetic redundancy fuels polygenic adaptation in *Drosophila*. *PLoS Biol.* **17**, e3000128. doi:10.1371/journal.pbio.3000128

Barton, B. T. and Schmitz, O. J. (2009). Experimental warming transforms multiple predator effects in a grassland food web. *Ecol. Lett.* **12**, 1317-1325. doi:10.1111/j.1461-0248.2009.01386.x

Bergland, A. O., Behrman, E. L., O'Brien, K. R., Schmidt, P. S. and Petrov, D. A. (2014). Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in *Drosophila*. *PLoS Genet.* **10**, 19. doi:10.1371/journal.pgen.1004775

Blackburn, S., van Heerwaarden, B., Kellermann, V. and Sgrò, C. M. (2014). Evolutionary capacity of upper thermal limits: beyond single trait assessments. *J. Exp. Biol.* **217**, 1918-1924. doi:10.1242/jeb.099184

Blanckenhorn, W. U. and Fairbairn, D. (1995). Life history adaptation along a latitudinal cline in the water strider *Aquarius remigis* (Heteroptera: Gerridae). *J. Evol. Biol.* **8**, 21-41. doi:10.1046/j.1420-9101.1995.8010021.x

Blows, M. W. and Hoffmann, A. A. (2005). A reassessment of the genetic limits to evolutionary change. *Ecology* **86**, 1371-1384. doi:10.1890/04-1209

Boggs, C. L. and Freeman, K. D. (2005). Larval food limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia* **144**, 353-361. doi:10.1007/s00442-005-0076-6

Borer, E. T., Harpole, W. S., Adler, P. B., Lind, E. M., Orrock, J. L., Seabloom, E. W. and Smith, M. D. (2014). Finding generality in ecology: a model for globally distributed experiments. *Method. Ecol. Evol.* **5**, 65-73. doi:10.1111/2041-210X.12125

Bowler, K. and Kashmeery, A. (1979). Recovery from heat injury in the blowfly, *Calliphora erythrocephala*. *J. Therm. Biol.* **4**, 197-202. doi:10.1016/0306-4565(79)90001-9

Bowler, K. and Terblanche, J. S. (2008). Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biol. Rev.* **83**, 339-355. doi:10.1111/j.1469-185X.2008.00046.x

Bradshaw, W. and Holzapfel, C. (2008). Genetic response to rapid climate change: it's seasonal timing that matters. *Mol. Ecol.* **17**, 157-166. doi:10.1111/j.1365-294X.2007.03509.x

Brennan, R. S., Garrett, A. D., Huber, K. E., Hargarten, H. and Pespeni, M. H. (2019). Rare genetic variation and balanced polymorphisms are important for survival in global change conditions. *Proc. R. Soc. B Biol. Sci.* **286**, 20190943. doi:10.1098/rspb.2019.0943

Bridle, J. and Hoffmann, A. (2022). Understanding the biology of species' ranges: when and how does evolution change the rules of ecological engagement? *Phil. Trans. R. Soc. B Biol. Sci.* **377**, 20210027. doi:10.1098/rstb.2021.0027

Bridle, J. R., Buckley, J., Bodsworth, E. J. and Thomas, C. D. (2014). Evolution on the move: specialization on widespread resources associated with rapid range expansion in response to climate change. *Proc. R. Soc. B* **281**, 20131800. doi:10.1098/rspb.2013.1800

Bubli, O. A., Imasheva, A. G. and Loeschcke, V. (1998). Selection for knockdown resistance to heat in *Drosophila melanogaster* at high and low larval densities. *Evolution* **52**, 619-625. doi:10.2307/2411097

Bubliy, O. 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. doi:10.1111/j.1420-9101.2005.00928.x

Buckley, J. and Bridle, J. R. (2014). Loss of adaptive variation during evolutionary responses to climate change. *Ecol. Lett.* **17**, 1316-1325. doi:10.1111/ele.12340

Burke, M. K., Dunham, J. P., Shahrestani, P., Thornton, K. R., Rose, M. R. and Long, A. D. (2010). Genome-wide analysis of a long-term evolution experiment with *Drosophila*. *Nature* **467**, U111-U587. doi:10.1038/nature09352

Burny, C., Nolte, V., Dolezal, M. and Schlotterer, C. (2022). Genome-wide selection signatures reveal widespread synergistic effects of two different stressors in *Drosophila melanogaster*. *Proc. R. Soc. B Biol. Sci.* **289**, 20221857. doi:10.1098/rspb.2022.1857

Bush, A., Mokany, K., Catullo, R., Hoffmann, A., Kellermann, V., Sgrò, C., McEvey, S. and Ferrier, S. (2016). Incorporating evolutionary adaptation in species distribution modelling reduces projected vulnerability to climate change. *Ecol. Lett.* **19**, 1468-1478. doi:10.1111/ele.12696

Cassel-Lundhagen, A., Kaňuch, P., Low, M. and Berggren, Å. (2011). Limited gene flow may enhance adaptation to local optima in isolated populations of the Roesel's bush cricket (*Metrioptera roeselii*). *J. Evol. Biol.* **24**, 381-390. doi:10.1111/j.1420-9101.2010.02174.x

Cavicchi, S., Guerra, D., Latorre, V. and Huey, R. B. (1995). Chromosomal analysis of heat-shock tolerance in *Drosophila melanogaster* evolving at different temperatures in the laboratory. *Evolution* **49**, 676-684. doi:10.1111/j.1558-5646.1995.tb02304.x

Cheng, C., Tan, J. C., Hahn, M. W. and Besansky, N. J. (2018). Systems genetic analysis of inversion polymorphisms in the malaria mosquito *Anopheles gambiae*. *Proc. Natl. Acad. Sci. USA* **115**, E7005-E7014. doi:10.1073/pnas.1806760115

Chown, S. L. and Gaston, K. J. (2010). Body size variation in insects: a macroecological perspective. *Biol. Rev.* **85**, 139-169. doi:10.1111/j.1469-185X.2009.00097.x

Clusella-Trullas, S. and Nielsen, M. (2020). The evolution of insect body coloration under changing climates. *Curr. Opin. Insect Sci.* **41**, 25-32. doi:10.1016/j.cois.2020.05.007

Colinet, H., Sinclair, B. J., Vernon, P. and Renault, D. (2015). Insects in fluctuating thermal environments. *Annu. Rev. Entomol.* **60**, 123-140. doi:10.1146/annurev-ento-010814-021017

Cushing, J. M. and Stefanko, K. (2021). A Darwinian dynamic model for the evolution of post-reproduction survival. *J. Biol. Syst.* **29**, 433-450. doi:10.1142/S0218339021400088

Daane, J. M., Auvinet, J., Stoebenau, A., Yergeau, D., Harris, M. P. and Detrich, H. W. (2020). Developmental constraint shaped genome evolution and erythrocyte loss in Antarctic fishes following paleoclimate change. *PLoS Genet.* **16**, e1009173. doi:10.1371/journal.pgen.1009173

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.* **18**, 838-846. doi:10.1111/j.1420-9101.2005.00914.x

Davis, A. J., Jenkinson, L. S., Lawton, J. H., Shorrocks, B. and Wood, S. (1998). Making mistakes when predicting shifts in species range in response to global warming. *Nature* **391**, 783-786. doi:10.1038/35842

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. Natl. Acad. Sci. USA* **105**, 6668-6672. doi:10.1073/pnas.0709472105

Diamond, S. E. (2017). Evolutionary potential of upper thermal tolerance: biogeographic patterns and expectations under climate change. *Ann. N. Y. Acad. Sci.* **1389**, 5-19. doi:10.1111/nyas.13223

Dierks, A., Kolzow, N., Franke, K. and Fischer, K. (2012). Does selection on increased cold tolerance in the adult stage confer resistance throughout development? *J. Evol. Biol.* **25**, 1650-1657. doi:10.1111/j.1420-9101.2012.02547.x

Donelson, J. M., Munday, P. L., McCormick, M. I. and Pitcher, C. R. (2012). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nat. Clim. Change* **2**, 30-32. doi:10.1038/nclimate1323

Elderkin, C. L., Stoeckel, J. A., Klerks, P. L. and Berg, D. J. (2004). Heritability of heat tolerance in zebra mussel Veligers. *J. Gt. Lakes Res.* **30**, 360-366. doi:10.1016/S0380-1330(04)70353-6

Esperk, T., Kjaersgaard, A., Walters, R. J., Berger, D. and Blanckenhorn, W. U. (2016). Plastic and evolutionary responses to heat stress in a temperate dung fly: negative correlation between basal and induced heat tolerance? *J. Evol. Biol.* **29**, 900-915. doi:10.1111/jeb.12832

Falconer, D. and Mackay, F. (1996). *Introduction to Quantitative Genetics*. Pearson Education.

Faull, K. J. and Williams, C. R. (2015). Intraspecific variation in desiccation survival time of *Aedes aegypti* (L.) mosquito eggs of Australian origin. *J. Vector Ecol.* **40**, 292-300. doi:10.1111/jvec.12167

Fedorka, K., Winterhalter, W., Shaw, K., Brogan, W. and Mousseau, T. (2012). The role of gene flow asymmetry along an environmental gradient in constraining local adaptation and range expansion. *J. Evol. Biol.* **25**, 1676-1685. doi:10.1111/j.1420-9101.2012.02552.x

Freda, P. J., Ali, Z. M., Heter, N., Ragland, G. J. and Morgan, T. J. (2019). Stage-specific genotype-by-environment interactions for cold and heat hardiness in *Drosophila melanogaster*. *Heredity* **123**, 479-491. doi:10.1038/s41437-019-0236-9

Geerts, A., Vanoverbeke, J., Vanschoenwinkel, B., Van Doorslaer, W., Feuchtmayr, H., Atkinson, D., Moss, B., Davidson, T. A., Sayer, C. D. and De Meester, L. (2015). Rapid evolution of thermal tolerance in the water flea *Daphnia*. *Nat. Clim. Change* **5**, 665-668. doi:10.1038/nclimate2628

Gilchrist, G. W. and Huey, R. B. (1999). The direct response of *Drosophila melanogaster* to selection on knockdown temperature. *Heredity* **83**, 15-29. doi:10.1038/sj.hdy.6885330

Gilchrist, G. W., Huey, R. B. and Partridge, L. (1997). Thermal sensitivity of *Drosophila melanogaster*: evolutionary responses of adults and eggs to laboratory natural selection at different temperatures. *Physiol. Zool.* **70**, 403-414. doi:10.1086/515853

Gotcha, N., Machekano, H., Cuthbert, R. N. and Nyamukondiwa, C. (2021). Heat tolerance may determine activity time in coprophagic beetle species (Coleoptera: Scarabaeidae). *Insect Science* **28**, 1076-1086. doi:10.1111/1744-7917.12844

Griffin, P. C., Hangartner, S. B., Fournier-Level, A. and Hoffmann, A. A. (2017). Genomic trajectories to desiccation resistance: convergence and divergence among replicate selected *Drosophila* lines. *Genetics* **205**, 871-890. doi:10.1534/genetics.116.187104

Gu, X., Chen, W., Perry, T., Batterham, P. and Hoffmann, A. A. (2021). Genomic knockout of hsp23 both decreases and increases fitness under opposing thermal extremes in *Drosophila melanogaster*. *Insect Biochem. Mol. Biol.* **139**, 103652. doi:10.1016/j.ibmb.2021.103652

Gunderson, A. R. and Stillman, J. H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc. R. Soc. B* **282**, 20150401. doi:10.1098/rspb.2015.0401

Hahn, M. W., Han, M. V. and Han, S.-G. (2007). Gene family evolution across 12 *Drosophila* genomes. *PLoS Genet.* **3**, e197. doi:10.1371/journal.pgen.0030197

Hamann, E., Blevins, C., Franks, S. J., Jameel, M. I. and Anderson, J. T. (2021). Climate change alters plant-herbivore interactions. *New Phytol.* **229**, 1894-1910. doi:10.1111/nph.17036

Hangartner, S. and Hoffmann, A. A. (2016). Evolutionary potential of multiple measures of upper thermal tolerance in *Drosophila melanogaster*. *Funct. Ecol.* **30**, 442-452. doi:10.1111/1365-2435.12499

Hangartner, S., Dworkin, I., DeNieu, M. and Hoffmann, A. A. (2017). Does increased heat resistance result in higher susceptibility to predation? A test using *Drosophila melanogaster* selection and hardening. *J. Evol. Biol.* **30**, 1153-1164. doi:10.1111/jeb.13084

Hangartner, S., Lasne, C., Sgrò, C. M., Connallon, T. and Monro, K. (2020). Genetic covariances promote climatic adaptation in Australian *Drosophila*. *Evolution* **74**, 326-337. doi:10.1111/evo.13831

Harada, T., Takenaka, S., Maihara, S., Ito, K. and Tamura, T. (2011). Changes in life–history traits of the water strider *Aquarius paludum* in accordance with global warming. *Physiol. Entomol.* **36**, 309-316. doi:10.1111/j.1365-3032.2011.00798.x

Harshman, L. G. and Hoffmann, A. A. (2000). Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends Ecol. Evol.* **15**, 32-36. doi:10.1016/S0169-5347(99)01756-5

Hector, T. E., Hoang, K. L., Li, J. and King, K. C. (2022). Symbiosis and host responses to heating. *Trends Ecol. Evol.* **37**, 611-624. doi:10.1016/j.tree.2022.03.011

Hector, T. E., Sgro, C. M. and Hall, M. D. (2021). Thermal limits in the face of infectious disease: How important are pathogens? *Glob. Change Biol.* **27**, 4469-4480. doi:10.1111/gcb.15761

Herremans, M., Gielen, K., Vanormelingen, P., Veraghtert, W., Swinnen, K. R. R. and Maes, D. (2021). Abundant citizen science data reveal that the peacock butterfly aglais io recently became bivoltine in Belgium. *Insects* **12**, 683. doi:10.3390/insects12080683

Huey, R. B., Patridge, L. and Fowler, K. (1991). Thermal sensitivity of *Drosophila melanogaster* responds rapidly to laboratory natural selection. *Evolution* **45**, 751-756. doi:10.2307/2409925

Huey, R., Crill, W., Kingsolver, J. and Weber, K. (1992). A method for rapid measurement of heat or cold resistance of small insects. *Funct. Ecol.* **6**, 489-494. doi:10.2307/2389288

Hiddink, J. G., Burrows, M. T. and García Molinos, J. (2015). Temperature tracking by North Sea benthic invertebrates in response to climate change. *Glob. Change Biol.* **21**, 117-129. doi:10.1111/gcb.12726

Hill, M. P., Chown, S. L. and Hoffmann, A. A. (2013). A predicted niche shift corresponds with increased thermal resistance in an invasive mite, *Halotydeus destructor*. *Glob. Ecol. Biogeogr.* **22**, 942-951. doi:10.1111/geb.12059

Hoffmann, A. A. and Blows, M. W. (1994). Species borders: ecological and evolutionary perspectives. *Trends Ecol. Evol.* **9**, 223-227. doi:10.1016/0169-5347(94)90248-8

Hoffmann, A. A. and Bridle, J. (2022). The dangers of irreversibility in an age of increased uncertainty: revisiting plasticity in invertebrates. *Oikos* **2022**, e08715. doi:10.1111/oik.08715

Hoffmann, A. A. and Merila, J. (1999). Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol. Evol.* **14**, 96-101. doi:10.1016/S0169-5347(99)01595-5

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

Hoffmann, A. A. and Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature* **470**, 479-485. doi:10.1038/nature09670

Hoffmann, A. A. and Sgrò, C. M. (2018). Comparative studies of critical physiological limits and vulnerability to environmental extremes in small ectotherms: How much environmental control is needed? *Integr. Zool.* **13**, 355-371. doi:10.1111/1749-4877.12297

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. doi:10.1016/S0022-1910(96)00108-4

Hoffmann, A. A., Hallas, R., Sinclair, C. and Partridge, L. (2001). Rapid loss of stress resistance in *Drosophila melanogaster* under adaptation to laboratory culture. *Evolution* **55**, 436-438. doi:10.1111/j.0014-3820.2001.tb01305.x

Hoffmann, A., Hallas, R., Dean, J. and Schiffer, M. (2003a). Low potential for climatic stress adaptation in a rainforest species. *Science* **301**, 100-102. doi:10.1126/science.1084296

Hoffmann, A. A., Sorensen, J. G. and Loeschcke, V. (2003b). Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Therm. Biol.* **28**, 175-216. doi:10.1016/S0306-4565(02)00057-8

Hoffmann, A. A., Chown, S. L. and Clusella-Trullas, S. (2013). Upper thermal limits in terrestrial ectotherms: How constrained are they?. *Funct. Ecol.* **27**, 934-949. doi:10.1111/j.1365-2435.2012.02036.x

Huey, R. B., Kearney, M. R., Krockenberger, A., Holtum, J. A. M., Jess, M. and Williams, S. E. (2012). Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Phil. Trans. R. Soc. B Biol. Sci.* **367**, 1665-1679. doi:10.1098/rstb.2012.0005

Husby, A., Visser, M. E. and Kruuk, L. E. B. (2011). Speeding up microevolution: The effects of increasing temperature on selection and genetic variance in a wild bird population. *PLoS Biol.* **9**, e1000585. doi:10.1371/journal.pbio.1000585

James, A. C., Azevedo, R. B. R. and Partridge, L. (1995). Cellular basis and developmental timing in a size cline of *Drosophila melanogaster*. *Genetics* **140**, 659-666. doi:10.1093/genetics/140.2.659

Jensen, D., Overgaard, J. and Sørensen, J. G. (2007). The influence of developmental stage on cold shock resistance and ability to cold-harden in *Drosophila melanogaster*. *J. Insect Physiol.* **53**, 179-186. doi:10.1016/j.jinsphys.2006.11.008

Johnstone, M., Schiffer, M. and Hoffmann, A. A. (2017). Separating multiple sources of variation on heat resistance in *Drosophila hydei*. *J. Insect Physiol.* **96**, 122-127. doi:10.1016/j.jinsphys.2016.11.001

Jørgensen, L. B., Malte, H. and Overgaard, J. (2019). How to assess *Drosophila* heat tolerance: Unifying static and dynamic tolerance assays to predict heat distribution limits. *Funct. Ecol.* **33**, 629-642. doi:10.1111/1365-2435.13279

Jørgensen, L. B., Malte, H., Ørsted, M., Klahn, N. A. and Overgaard, J. (2021). A unifying model to estimate thermal tolerance limits in ectotherms across static, dynamic and fluctuating exposures to thermal stress. *Sci. Rep.* **11**, 12840. doi:10.1038/s41598-021-92004-6

Jørgensen, L. B., Ørsted, M., Malte, H., Wang, T. and Overgaard, J. (2022). Extreme escalation of heat failure rates in ectotherms with global warming. *Nature*, **661**, 83-91. doi:10.1038/s41586-022-05334-4

Kawecki, T. J., Lenski, R. E., Ebert, D., Hollis, B., Olivieri, I. and Whitlock, M. C. (2012). Experimental evolution. *Trends Ecol. Evol.* **27**, 547-560. doi:10.1016/j.tree.2012.06.001

Kearney, M., Porter, W. P., Williams, C., Ritchie, S. and Hoffmann, A. A. (2009). Integrating biophysical models and evolutionary theory to predict climatic impacts on species' ranges: the dengue mosquito Aedes aegypti in Australia. *Funct. Ecol.* **23**, 528-538. doi:10.1111/j.1365-2435.2008.01538.x

Kellermann, V. and Sgrò, C. M. (2018). Evidence for lower plasticity in CT_{MAX} at warmer developmental temperatures. *J. Evol. Biol.* **31**, 1300-1312. doi:10.1111/jeb.13303

Kellermann, V. M., van Heerwaarden, B., Hoffmann, A. A. and Sgrò, C. M. (2006). Very low additive genetic variance and evolutionary potential in multiple populations of two rainforest *Drosophila* species. *Evolution* **60**, 1104-1108. doi:10.1554/05-710.1

Kellermann, V., 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. doi:10.1126/science.1175443

Kellermann, V., Overgaard, J., Hoffmann, A. A., Fløjgaard, C., Svenning, J.-C. and Loeschcke, V. (2012). Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. *Proc. Natl. Acad. Sci. USA* **109**, 16228-16233. doi:10.1073/pnas.1207553109

Kelly, M. W., Sanford, E. and Grosberg, R. K. (2012). Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proc. R. Soc. B* **279**, 349-356. doi:10.1098/rspb.2011.0542

Ketola, T., Kellermann, V., Kristensen, T. N. and Loeschcke, V. (2012). Constant, cycling, hot and cold thermal environments: strong effects on mean viability but not on genetic estimates. *J. Evol. Biol.* **25**, 1209-1215. doi:10.1111/j.1420-9101.2012.02513.x

King, O. D. and Masel, J. (2007). The evolution of bet-hedging adaptations to rare scenarios. *Theor. Popul. Biol.* **72**, 560-575. doi:10.1016/j.tpb.2007.08.006

Kingsolver, J. G. and Buckley, L. B. (2015). Climate variability slows evolutionary responses of *Colias* butterflies to recent climate change. *Proc. R. Soc. B* **282**, 20142470. doi:10.1098/rspb.2014.2470

Kingsolver, J. G. and Buckley, L. B. (2020). Ontogenetic variation in thermal sensitivity shapes insect ecological responses to climate change. *Curr. Opin. Insect Sci.* **41**, 17-24. doi:10.1016/j.cois.2020.05.005

Kingsolver, J. G. and Huey, R. B. (2008). Size, temperature, and fitness: three rules. *Evol. Ecol. Res.* **10**, 251-268.

Kingsolver, J. G., Woods, H. A., Buckley, L. B., Potter, K. A., MacLean, H. J. and Higgins, J. K. (2011). Complex life cycles and the responses of insects to climate change. *Integr. Comp. Biol.* 51, 719-732. doi:10.1093/icb/icr015

Klockmann, M. and Fischer, K. (2017). Effects of temperature and drought on early life stages in three species of butterflies: Mortality of early life stages as a key determinant of vulnerability to climate change? *Ecol. Evol.* 7, 10871-10879. doi:10.1002/ece3.3588

Krebs, R. A. and Feder, M. E. (1997). Natural variation in the expression of the heat–shock protein HSP70 in a population of *Drosophila melanogaster* and its correlation with tolerance of ecologically relevant thermal stress. *Evolution* 51, 173-179. doi:10.1111/j.1558-5646.1997.tb02398.x

Krebs, R. A. and Thompson, K. A. (2006). Direct and correlated effects of selection on flight after exposure to thermal stress in *Drosophila melanogaster*. *Genetica* 128, 217-225. doi:10.1007/s10709-005-5704-z

Kristensen, T. N., Overgaard, J., Lassen, J., Hoffmann, A. A. and Sgrò, C. (2015). Low evolutionary potential for egg-to-adult viability in *Drosophila melanogaster* at high temperatures. *Evolution* 69, 803-814. doi:10.1111/evo. 12617

Langmuller, A. M. and Schlotterer, C. (2020). Low concordance of short-term and long-term selection responses in experimental *Drosophila* populations. *Mol. Ecol.* 29, 3466-3475. doi:10.1111/mec.15579

Langmuller, A. M., Dolezal, M. and Schlotterer, C. (2021). Fine mapping without phenotyping: Identification of selection targets in secondary evolve and resequence experiments. *Genome Biol. Evol.* 13, evab154. doi:10.1093/gbe/evab154

Levy, O., Buckley, L. B., Keitt, T. H., Smith, C. D., Boateng, K. O., Kumar, D. S. and Angilletta, M. J. (2015). Resolving the life cycle alters expected impacts of climate change. *Proc. R. Soc. B Biol. Sci.* 282, 20150837. doi:10.1098/rspb.2015. 0837

Li, F., Rane, R. V., Luria, V., Xiong, Z., Chen, J., Li, Z., Catullo, R. A., Griffin, P. C., Schiffer, M. and Pearce, S. (2022). Phylogenomic analyses of the genus *Drosophila* reveals genomic signals of climate adaptation. *Mol. Ecol. Resour.* 22, 1559-1581. doi:10.1111/1755-0998.13561

Loeschcke, V. and Krebs, R. A. (1996). Selection for heat-shock resistance in larval and in adult *Drosophila buzzattii:* Comparing direct and indirect responses. *Evolution* 50, 2354-2359. doi:10.1111/j.1558-5646.1996.tb03623.x

Ma, F. Z., Lü, Z. C., Wang, R. and Wan, F. H. (2014). Heritability and evolutionary potential in thermal tolerance traits in the invasive Mediterranean cryptic species of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *PLoS ONE* 9, e103279. doi:10.1371/journal.pone.0103279

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. doi:10.1046/j.1420-9101.2002.00439.x

Maino, J. L., Kong, J. D., Hoffmann, A. A., Barton, M. G. and Kearney, M. R. (2016). Mechanistic models for predicting insect responses to climate change. *Curr. Opin. Insect Sci.* 17, 81-86. doi:10.1016/j.cois.2016.07.006

Marshall, D. and Uller, T. (2007). When is a maternal effect adaptive? *Oikos* 116, 1957-1963. doi:10.1111/j.2007.0030-1299.16203.x

Masaki, S. (1980). Summer diapause. *Annu. Rev. Entomol.* 25, 1-25. doi:10.1146/annurev.en.25.010180.000245

Mauro, A. A., Torres-Dowdall, J., Marshall, C. A. and Ghalambor, C. K. (2021). A genetically based ecological trade-off contributes to setting a geographic range limit. *Ecol. Lett.* 24, 2739-2749. doi:10.1111/ele.13900

McAfee, A., Chapman, A., Higo, H., Underwood, R., Milone, J., Foster, L. J., Guarna, M. M., Tarpy, D. R. and Pettis, J. S. (2020). Vulnerability of honey bee queens to heat-induced loss of fertility. *Nat. Sustain.* 3, 367-376. doi:10.1038/s41893-020-0493-x

McColl, G., Hoffmann, A. A. and McKechnie, S. W. (1996). Response of two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*. *Genetics* 143, 1615-1627. doi:10.1093/genetics/143.4.1615

McGuigan, K., Van Homrigh, A. and Blows, M. W. (2008). An evolutionary limit to male mating success. *Evolution* 62, 1528-1537. doi:10.1111/j.1558-5646.2008. 00379.x

McGuigan, K., Hoffmann, A. A. and Sgrò, C. M. (2021). How is epigenetics predicted to contribute to climate change adaptation? What evidence do we need? *Philos. Trans. R. Soc. B Biol. Sci.* 376, 20200119. doi:10.1098/rstb.2020.0119

Menke, S. B., Harte, J. and Dunn, R. R. (2014). Changes in ant community composition caused by 20 years of experimental warming vs. 13 years of natural climate shift. *Ecosphere* 5, 1-17. doi:10.1890/ES13-00234.1

Mesas, A., Jaramillo, A. and Castañeda, L. E. (2021). Experimental evolution on heat tolerance and thermal performance curves under contrasting thermal selection in *Drosophila subobscura*. *J. Evol. Biol.* 34, 767-778. doi:10.1111/jeb. 13777

Michalak, P., Kang, L., Schou, M. F., Garner, H. R. and Loeschcke, V. (2019). Genomic signatures of experimental adaptive radiation in *Drosophila*. *Mol. Ecol.* 28, 600-614. doi:10.1111/mec.14917

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.* 24, 694-700. doi:10.1111/j.1365-2435.2009.01666.x

Mitchell, K. A., Sinclair, B. J. and Terblanche, J. S. (2013). Ontogenetic variation in cold tolerance plasticity in *Drosophila*: is the Bogert effect bogus? *Naturwissenschaften* 100, 281-284. doi:10.1007/s00114-013-1023-8

Moghadam, N. N., Ketola, T., Pertoldi, C., Bahrndorff, S. and Kristensen, T. N. (2019). Heat hardening capacity in *Drosophila melanogaster* is life stage-specific and juveniles show the highest plasticity. *Biol. Lett.* 15, 20180628. doi:10.1098/rsbl.2018.0628

Morrison, W. W. and Milkman, R. (1978). Modification of heat resistance in *Drosophila* by selection. *Nature* 273, 49-50. doi:10.1038/273049b0

Nespolo, R. F., Roff, D. A. and Fairbairn, D. J. (2008). Energetic trade-off between maintenance costs and flight capacity in the sand cricket (*Gryllus firmus*). *Funct. Ecol.* 22, 624-631. doi:10.1111/j.1365-2435.2008.01394.x

Netherer, S. and Schopf, A. (2010). Potential effects of climate change on insect herbivores in European forests—General aspects and the pine processionary moth as specific example. *For. Ecol. Manag.* 259, 831-838. doi:10.1016/j.foreco. 2009.07.034

Nguyen, T. M., Bressac, C. and Chevrier, C. (2013). Heat stress affects male reproduction in a parasitoid wasp. *J. Insect Physiol.* 59, 248-254. doi:10.1016/j. jinsphys.2012.12.001

Nielsen, M. E. and Kingsolver, J. G. (2020). Compensating for climate change–induced cue–environment mismatches: evidence for contemporary evolution of a photoperiodic reaction norm in *Colias* butterflies. *Ecol. Lett.* 23, 1129-1136. doi:10.1111/ele.13515

O'Brien, E. K., Walter, G. M. and Bridle, J. (2022). Environmental variation and biotic interactions limit adaptation at ecological margins: lessons from rainforest *Drosophila* and European butterflies. *Philos. Trans. R. Soc. B* 377, 20210017. doi:10.1098/rstb.2021.0017

Ørsted, M., Hoffmann, A. A., Rohde, P. D., Sorensen, P. and Kristensen, T. N. (2019). Strong impact of thermal environment on the quantitative genetic basis of a key stress tolerance trait. *Heredity* 122, 315-325. doi:10.1038/s41437-018-0117-7

Ørsted, M., Jørgensen, L. B. and Overgaard, J. (2022). Finding the right thermal limit: a framework to reconcile ecological, physiological and methodological aspects of CT_{max} in ectotherms. *J. Exp. Biol.* 225, jeb244514. doi:10.1242/jeb. 244514

Otte, K. A., Nolte, V., Mallard, F. and Schlotterer, C. (2021). The genetic architecture of temperature adaptation is shaped by population ancestry and not by selection regime. *Genome Biol.* 22, 211. doi:10.1186/s13059-021-02425-9

Overgaard, J., Kearney, M. R. and Hoffmann A. A. (2014). Sensitivity to thermal extremes in Australian *Drosophila* implies similar impacts of climate change on the distribution of widespread and tropical species. *Glob. Change Biol.* 20, 1738-1750. doi:10.1111/gcb.12521

Pandori, L. L. M. and Sorte, C. J. B. (2019). The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. *Oikos* 128, 621-629. doi:10. 1111/oik.05886

Parratt, S. R., Walsh, B. S., Metelmann, S., White, N., Manser, A., Bretman, A. J., Hoffmann, A. A., Snook, R. R. and Price, T. A. R. (2021). Temperatures that sterilize males better match global species distributions than lethal temperatures. *Nat. Clim. Change* 11, 481-484. doi:10.1038/s41558-021-01047-0

Phillips, M. A., Kutch, I. C., Long, A. D. and Burke, M. K. (2020). Increased time sampling in an evolve-and-resequence experiment with outcrossing *Saccharomyces cerevisiae* reveals multiple paths of adaptive change. *Mol. Ecol.* 29, 4898-4912. doi:10.1111/mec.15687

Phillips, M. A., Kutch, I. C., McHugh, K. M., Taggard, S. K. and Burke, M. K. (2021). Crossing design shapes patterns of genetic variation in synthetic recombinant populations of *Saccharomyces cerevisiae*. *Sci. Rep.* 11, 19551. doi:10.1038/s41598-021-99026-0

Pincebourde, S. and Casas, J. (2015). Warming tolerance across insect ontogeny: influence of joint shifts in microclimates and thermal limits. *Ecology* 96, 986-997. doi:10.1890/14-0744.1

Pottier, P., Burke, S., Zhang, R. Y., Noble, D. W. A., Schwanz, L. E., Drobniak, S. M. and Nakagawa, S. (2022). Developmental plasticity in thermal tolerance: Ontogenetic variation, persistence, and future directions. *Ecol. Lett.* 25, 2245-2268. doi:10.1111/ele.14083

Pruisscher, P., Nylin, S., Gotthard, K. and Wheat, C. W. (2018). Genetic variation underlying local adaptation of diapause induction along a cline in a butterfly. *Mol. Ecol.* 27, 3613-3626. doi:10.1111/mec.14829

Radchuk, V., Turlure, C. and Schtickzelle, N. (2013). Each life stage matters: the importance of assessing the response to climate change over the complete life cycle in butterflies. *J. Anim. Ecol.* 82, 275-285. doi:10.1111/j.1365-2656.2012. 02029.x

Rane, R. V., Pearce, S. L., Li, F., Coppin, C., Schiffer, M., Shirriffs, J., Sgrò, C. M., Griffin, P. C., Zhang, G., Lee, S. F. et al. (2019). Genomic changes associated with adaptation to arid environments in cactophilic *Drosophila* species. *BMC Genomics* 20, 52. doi:10.1186/s12864-018-5413-3

Rebolledo, A. P., Sgrò, C. M., Monro, K. (2021). Thermal performance curves are shaped by prior thermal environment in early life. *Front. Physiol.* 12, 738338. doi:10.3389/fphys.2021.738338

Rezende, E. L., Castaneda, L. E. and Santos, M. (2014). Tolerance landscapes in thermal ecology. *Funct. Ecol.* 28, 799-809. doi:10.1111/1365-2435.12268

Rinehart, J. P., Yocum, G. D. and Denlinger, D. L. (2000). Thermotolerance and rapid cold hardening ameliorate the negative effects of brief exposures to high or low temperatures on fecundity in the flesh fly, *Sarcophaga crassipalpis*. *Physiol. Entomol.* **25**, 330-336. doi:10.1046/j.1365-3032.2000.00201.x

Robinet, C. and Roques, A. (2010). Direct impacts of recent climate warming on insect populations. *Integr. Zool.* **5**, 132-142. doi:10.1111/j.1749-4877.2010.00196.x

Roff, D. A. (1992). *The Evolution of Life Histories*. London: Chapman and Hall.

Roff, D. A. and Fairbairn, D. J. (2007a). The evolution of trade-offs: where are we? *J. Evol. Biol.* **20**, 433-447. doi:10.1111/j.1420-9101.2006.01255.x

Roff, D. A. and Fairbairn, D. J. (2007b). Laboratory evolution of the migratory polymorphism in the sand cricket: combining physiology with quantitative genetics. *Physiol. Biochem. Zool.* **80**, 358-369. doi:10.1086/518012

Rudman, S. M., Greenblum, S. I., Rajpurohit, S., Betancourt, N. J., Hanna, J., Tilk, S., Yokoyama, T., Petrov, D. A. and Schmidt, P. (2022). Direct observation of adaptive tracking on ecological time scales in *Drosophila*. *Science* **375**, 1246. doi:10.1126/science.abj7484

Ruel, J. J. and Ayres, M. P. (1999). Jensen's inequality predicts effects of environmental variation. *Trends Ecol. Evol.* **14**, 361-366. doi:10.1016/S0169-5347(99)01664-X

Sales, K., Vasudeva, R., Dickinson, M. E., Godwin, J. L., Lumley, A. J., Michalczyk, L., Hebberecht, L., Thomas, P., Franco, A. and Gage, M. J. G. (2018). Experimental heatwaves compromise sperm function and cause transgenerational damage in a model insect. *Nat. Commun.* **9**, 4771. doi:10.1038/s41467-018-07273-z

Sallé, A., Nageleisen, L. M. and Lieutier, F. (2014). Bark and wood boring insects involved in oak declines in Europe: Current knowledge and future prospects in a context of climate change. *For. Ecol. Manag.* **328**, 79-93. doi:10.1016/j.foreco.2014.05.027

Santos, M., Borash, D. J., Joshi, A., Bounlutay, N. and Mueller, L. D. (1997). Density-dependent natural selection in *Drosophila*: Evolution of growth rate and body size. *Evolution* **51**, 420-432. doi:10.1111/j.1558-5646.1997.tb02429.x

Sasaki, M. C. and Dam, H. G. (2021). Negative relationship between thermal tolerance and plasticity in tolerance emerges during experimental evolution in a widespread marine invertebrate. *Evol. Appl.* **14**, 2114-2123. doi:10.1111/eva.13270

Schaum, C. E., Buckling, A., Smirnoff, N., Studholme, D. J. and Yvon-Durocher, G. (2018). Environmental fluctuations accelerate molecular evolution of thermal tolerance in a marine diatom. *Nat. Commun.* **9**, 1719. doi:10.1038/s41467-018-03906-5

Schiffer, M., Hangartner, S. and Hoffmann, A. A. (2013). Assessing the relative importance of environmental effects, carry-over effects and species differences in thermal stress resistance: a comparison of Drosophilids across field and laboratory generations. *J. Exp. Biol.* **216**, 3790-3798. doi:10.1242/jeb.085126

Schluter, D., Price, T. D. and Rowe, L. (1991). Conflicting selection pressures and life-history trade-offs. *Proc. R. Soc. Lond. B Biol. Sci.* **246**, 11-17. doi:10.1098/rspb.1991.0118

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. doi:10.1111/j.0014-3820.2006.tb00505.x

Schneider, D., Ramos, A. G. and Cordoba-Aguilar, A. (2020). Multigenerational experimental simulation of climate change on an economically important insect pest. *Ecol. Evol.* **10**, 12893-12909. doi:10.1002/ece3.6847

Schou, M., Kristensen, T., Kellermann, V. and Loeschcke, V. (2014). A *Drosophila* laboratory evolution experiment points to low evolutionary potential under increased temperatures likely to be experienced in the future. *J. Evol. Biol.* **27**, 1859-1868. doi:10.1111/jeb.12436

Sgrò, C. M. and Hoffmann, A. A. (2004). Genetic correlations, tradeoffs and environmental variation. *Heredity* **93**, 241-248. doi:10.1038/sj.hdy.6800532

Sgrò, C. M., Overgaard, J., Kristensen, T., Mitchell, K., Cockerell, F. and Hoffmann, A. A. (2010). A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of *Drosophila melanogaster* from eastern Australia. *J. Evol. Biol.* **23**, 2484-2493. doi:10.1111/j.1420-9101.2010.02110.x

Sgrò, C. M., Terblanche, J. S. and Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annu. Rev. Entomol.* **61**, 433-451. doi:10.1146/annurev-ento-010715-023859

Sikkink, K. L., Ituarte, C. M., Reynolds, R. M., Cresko, W. A. and Phillips, P. C. (2014). The transgenerational effects of heat stress in the nematode *Caenorhabditis remanei* are negative and rapidly eliminated under direct selection for increased stress resistance in larvae. *Genomics* **104**, 438-446. doi:10.1016/j.ygeno.2014.09.014

Singer, M. C. and Parmesan, C. (2010). Phenological asynchrony between herbivorous insects and their hosts: signal of climate change or pre-existing adaptive strategy? *Philos. Trans. R. Soc. B Biol. Sci.* **365**, 3161-3176. doi:10.1098/rstb.2010.0144

Singh, P., van Bergen, E., Brattstrom, O., Osbaldeston, D., Brakefield, P. M. and Oostra, V. (2020). Complex multi-trait responses to multivariate environmental cues in a seasonal butterfly. *Evol. Ecol.* **34**, 713-734. doi:10.1007/s10682-020-10062-0

Speights, C. J., Harmon, J. P. and Barton, B. T. (2017). Contrasting the potential effects of daytime versus nighttime warming on insects. *Curr. Opin. Insect Sci.* **23**, 1-6. doi:10.1016/j.cois.2017.06.005

Stadelmann, G., Bugmann, H., Wermelinger, B., Meier, F. and Bigler, C. (2013). A predictive framework to assess spatio–temporal variability of infestations by the European spruce bark beetle. *Ecography* **36**, 1208-1217. doi:10.1111/j.1600-0587.2013.00177.x

Stephanou, G. and Alahiotis, S. (1983). Non-Mendelian inheritance of" heat-sensitivity" in *Drosophila melanogaster*. *Genetics* **103**, 93-107. doi:10.1093/genetics/103.1.93

Storch, D., Menzel, L., Frickenhaus, S. and Pörtner, H. O. (2014). Climate sensitivity across marine domains of life: limits to evolutionary adaptation shape species interactions. *Glob. Change Biol.* **20**, 3059-3067. doi:10.1111/gcb.12645

Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T. and Huey, R. B. (2014). Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 5610-5615. doi:10.1073/pnas.1316145111

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* **274**, 2935-2943. doi:10.1098/rspb.2007.0985

Thompson, R. M., Beardall, J., Beringer, J., Grace, M. and Sardina, P. (2013). Means and extremes: building variability into community-level climate change experiments. *Ecol. Lett.* **16**, 799-806. doi:10.1111/ele.12095

Udaka, H., Ueda, C. and Goto, S. G. (2010). Survival rate and expression of Heat-shock protein 70 and Frost genes after temperature stress in *Drosophila melanogaster* lines that are selected for recovery time from temperature coma. *J. Insect Physiol.* **56**, 1889-1894. doi:10.1016/j.jinsphys.2010.08.008

Uller, T., Nakagawa, S. and English, S. (2013). Weak evidence for anticipatory parental effects in plants and animals. *J. Evol. Biol.* **26**, 2161-2170. doi:10.1111/jeb.12212

van Asch, M., Salis, L., Holleman, L. J. M., van Lith, B. and Visser, M. E. (2013). Evolutionary response of the egg hatching date of a herbivorous insect under climate change. *Nat. Clim. Change* **3**, 244-248. doi:10.1038/nclimate1717

van Heerwaarden, B. and Kellermann, V. (2020). Does plasticity trade off with basal heat tolerance? *Trends Ecol. Evol.* **35**, 874-885. doi:10.1016/j.tree.2020.05.006

van Heerwaarden, B. and Sgrò, C. M. (2011). The effect of developmental temperature on the genetic architecture underlying size and thermal clines in *Drosophila melanogaster* and *D. simulans* from the east coast of Australia. *Evolution* **65**, 1048-1067. doi:10.1111/j.1558-5646.2010.01196.x

van Heerwaarden, B. and Sgrò, C. M. (2013). Multivariate analysis of adaptive capacity for upper thermal limits in *Drosophila simulans*. *J. Evol. Biol.* **26**, 800-809. doi:10.1111/jeb.12090

van Heerwaarden, B. and Sgrò, C. M. (2014). Is adaptation to climate change really constrained in niche specialists? *Proc. R. Soc. B Biol. Sci.* **281**, 20140396. doi:10.1098/rspb.2014.0396

van Heerwaarden, B. and Sgrò, C. M. (2021). Male fertility thermal limits predict vulnerability to climate warming. *Nat. Commun.* **12**, 2214. doi:10.1038/s41467-021-22546-w

van Heerwaarden, B., Kellermann, V., Schiffer, M., Blacket, M., Sgrò, 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* **276**, 1517-1526. doi:10.1098/rspb.2008.1288

van Heerwaarden, B., Lee, R. F. H., Wegener, B., Weeks, A. R. and Sgrò, C. M. (2012). Complex patterns of local adaptation in heat tolerance in *Drosophila simulans* from eastern Australia. *J. Evol. Biol.* **25**, 1765-1778. doi:10.1111/j.1420-9101.2012.02564.x

van Heerwaarden, B., Malmberg, M. and Sgrò, C. M. (2016). Increases in the evolutionary potential of upper thermal limits under warmer temperatures in two rainforest *Drosophila* species. *Evolution* **70**, 456-464. doi:10.1111/evo.12843

Verheyen, J., Tüzün, N. and Stoks, R. (2019). Using natural laboratories to study evolution to global warming: contrasting altitudinal, latitudinal, and urbanization gradients. *Curr. Opin. Insect Sci.* **35**, 10-19. doi:10.1016/j.cois.2019.06.001

Waite, H. R. and Sorte, C. J. B. (2022). Negative carry-over effects on larval thermal tolerances across a natural thermal gradient. *Ecology* **103**, e03565. doi:10.1002/ecy.3565

Walsh, B. and Blows, M. W. (2009). Abundant genetic variation plus strong selection=multivariate genetic constraints: A geometric view of adaptation. *Ann. Rev. Ecol. Evol. Syst.* **40**, 41-59. doi:10.1146/annurev.ecolsys.110308.120232

Walsh, B. S., Parratt, S. R., Hoffmann, A. A., Atkinson, D., Snook, R. R., Bretman, A. and Price, T. A. R. (2019). The impact of climate change on fertility. *Trends Ecol. Evol.* **34**, 249-259. doi:10.1016/j.tree.2018.12.002

Walsh, B. S., Parratt, S. R., Snook, R. R., Bretman, A., Atkinson, D. and Price, T. A. R. (2022). Female fruit flies cannot protect stored sperm from high temperature damage. *J. Therm. Biol.* **105**, 103209. doi:10.1016/j.jtherbio.2022.103209

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. doi:10.1371/journal.pbio.0040216

Zhang, W., Chang, X.-Q., Hoffmann, A., Zhang, S. and Ma, C.-S. (2015). Impact of hot events at different developmental stages of a moth: the closer to adult stage, the less reproductive output. *Sci. Rep.* **5**, 1-9. doi:10.9734/JSRR/2015/14076

Zhao, F., Zhang, W., Hoffmann, A. A. and Ma, C.-S. (2014). Night warming on hot days produces novel impacts on development, survival and reproduction in a small arthropod. *J. Anim. Ecol.* **83**, 769-778. doi:10.1111/1365-2656.12196

Zhao, F., Xing, K., Hoffmann, A. A. and Ma, C.-S. (2019). The importance of timing of heat events for predicting the dynamics of aphid pest populations. *Pest Manag. Sci.* **75**, 1866-1874. doi:10.1002/ps.5344

Zhu, L., Hoffmann, A. A., Li, S. M. and Ma, C.-S. (2021). Extreme climate shifts pest dominance hierarchy through thermal evolution and transgenerational plasticity. *Funct. Ecol.* **35**, 1524-1537. doi:10.1111/1365-2435.13774

Zvereva, E. L., Hunter, M. D., Zverev, V., Kruglova, O. Y. and Kozlov, M. V. (2019). Climate warming leads to decline in frequencies of melanic individuals in subarctic leaf beetle populations. *Sci. Total Environ.* **673**, 237-244. doi:10.1016/j.scitotenv.2019.03.458

Zwoinska, M. K., Rodrigues, L. R., Slate, J. and Snook, R. R. (2020). Phenotypic responses to and genetic architecture of sterility following exposure to sub-lethal temperature during development. *Front. Genet.* **11**, 573. doi:10.3389/fgene.2020.00573