Adaptation under climate change

Thesis submitted by

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Title page photograph: *Drosophila birchii* in the wild, photo credited to Dr Andrew Weeks.

Acknowledgments

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Contributions of others

This thesis was co-supervised by Megan Higgie and Conrad Hoskin, both of which are associated with James Cook University in the College of Science and Engineering. Both provided significant advice on experimental design, analysis, and writing throughout my project and are co-authors on all of my chapters. Both have a background in ecology and evolutionary biology and provided expertise in general concepts and importance of each study. Megan provided expertise in working with and maintaining *Drosophila* in the laboratory, specifically providing input into the *Drosophila* species used in this study. Megan also provided thoughtful conversations and knowledge in statistics and quantitative genetics.

Eleanor O'Brien provided expertise on quantitative genetics analyses and experimental design, and is a co-author on Chapter 2 and Chapter 5. Henry Stoetzel collected *Drosophila* wing photographs and landmarked the wings in Chapter 2. He also helped with counting eggs and is a co-author for Chapter 2.

The adjustable temperature arrays (Chapter 3) were a collaborative effort, but design and development was conducted by the JCU Innovation Centre. Wayne Morris designed the equipment and Russell Warburton wrote the operational coding for the equipment. Lexie Edwards provided outstanding technical support in building the temperature arrays and helped with design. As such, Wayne Morris, Russell Warburton, and Lexie Edwards are all co-authors on Chapter 3.

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Copyright statement

General abstract

Under environmental change, the persistence of a species centres on its ability to disperse from, or adapt to, the changing conditions. Many species lack the ability to disperse in a timeframe parallel to climate change and/or will be restricted by decreasing environmental space, so adaptation is necessary. Even more concerning than gradual climate change is that species will also have to contend with extreme heat events. Extreme heat events (heatwaves) are predicted to increase in frequency, intensity, and duration with climate change, and may pose an equal or greater risk to species than gradual climate change. My thesis examines mechanisms behind adaptation to climate change to shed light on how species may react to both gradual and sudden temperature rises. I used two closely-related forest species of *Drosophila* from the Australian Wet Tropics to examine how a generalist (*Drosophila serrata*) and a specialist (*Drosophila birchii*) species differ in their potential for adaptation.

First, I examined how genetic variance for life history and morphology traits differ between a benign and stressful thermal environment using a parent-offspring quantitative genetic design (Chapter 2). Genetic variation is a necessary prerequisite for selection and is known to change between environments. As such, determining how stressful thermal environments change genetic variance will be fundamental to predicting evolutionary potential. As fitness is often difficult to directly measure, I also assessed phenotypic and genetic covariances between fitness and two morphological traits to determine whether a morphological trait can be used as a proxy for fitness in the same environment, and how this relationship changes under stressful temperatures. My results showed that heritability of traits decreased from the benign to stressful environment; but that coefficients of genetic variance, and phenotypic and genetic covariances, show no consistent pattern of change across thermal regimes or between species. This is consistent with previous research and confirms that researchers will have to determine heritability values for fitness and morphological traits specific to their species and environment to accurately predict evolutionary potential to stressful temperatures.

Chapter 3 describes an adjustable temperature array that I designed, built, and validated for use in ecological and evolutionary studies on thermal physiology. The equipment allows for user-defined thermal environments across a temperature gradient with high accuracy and precision. It can implement both static and dynamic thermal regimes. In addition, it is modular and can be scaled to fit the user's needs to create individual thermal

landscapes for use with a variety of species and to answer a diverse amount of questions. This equipment forms an important component of my methodology for later experiments.

In Chapter 4, I looked at whether temperature preference, which is important in deciding both the rate and direction of adaptation, is co-adapted to key fitness traits and found evidence both for and against coadaptation. I measured productivity, development speed, and wing size along a thermal gradient to create thermal performance curves. I then used a similar methodology but allowed individuals to choose thermal environments to define temperature preference along this same gradient. By doing so, this incorporated oviposition preference site into the metric, making it one of the first studies to examine whether oviposition preference site is co-adapted to thermal fitness. I found that productivity is almost perfectly co-adapted to temperature preference, while evidence for coadaptation in development speed and wing size is lacking. Determining if temperature preference is co-adapted to certain fitness traits can allow researchers to use temperature preference as a proxy when fitness measures are unobtainable.

Next, I examined whether a heatwave can act as a selection event for thermal tolerance and whether this increases survival during a subsequent heatwave (Chapter 5). I also investigated how a heatwave impacts long-term fitness. I did so by creating artificially-affected heatwave populations of *D. birchii* by selecting for a highly-heritable thermal tolerance trait (static heat knockdown). I then tested survival during a subsequent 'heatwave' one year later and created thermal performance curves for productivity, development speed, and wing size. I found that surviving an initial heatwave does not future-proof a population against a subsequent heatwave. Further, I found that a heatwave decreases population-level fitness, and that fitness losses are worse with more intense heatwaves.

This thesis demonstrates that the heritability of key fitness traits may decrease with climate change; but that this is not consistent across species or populations. As such, researchers need to be thorough in using accurate heritability and genetic variance values when determining how species may respond to changing temperatures. This work also highlights the importance of behaviour in shaping a species' thermal niche and reveals that temperature preference may be used as a proxy when attempting to identify thermal optimums for species. Lastly, this study provides novel evidence that populations affected by a heatwave may not be better suited to surviving subsequent heatwaves and that heatwaves instead can cause maladaptation.

Overall, this research emphasizes that fitness, behaviour, and thermal tolerances are all important factors in surviving increasing temperatures but that each of these components

may contribute and/or be affected by gradual and sudden temperature rise in different ways. In summary, this research highlights the importance of taking a comprehensive approach when trying to predict whether a species can adapt to changing conditions.

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Chapter 1: General introduction

1.1 Climate change

Human-induced climate change may be the largest disturbance to ecological communities in this century (Thomas et al., 2004; Parmesan, 2006; Deutsch et al., 2008; Anjos & Toledo, 2018; Román-Palacios & Wiens, 2020). Changes associated with climate change are currently predicted to occur rapidly and at a scale too great for many species to overcome (Hoffmann & Sgrò, 2011). It is thought that this will result in population declines and probable extinctions in many vulnerable species (Parmesan, 2006; Warren et al., 2018; Román-Palacios & Wiens, 2020). The potential for a species to overcome climate change will depend on multiple factors—the amount of phenotypic plasticity in a population, the dispersal capability of a population, and the adaptive potential of a population can all contribute to species survival (Carlson et al., 2014; Merilä & Hendry, 2014; Catullo et al., 2019; Kelly, 2019). For many species, dispersal limitations and decreases in climatically-suitable habitat mean that adapting to changing environments will often be necessary for a species to persist (Hoffmann and Sgrò 2011; Carlson et al., 2014; Meester et al., 2018). However, the importance of adaptation for species survival under climate change has, until recently, been relatively neglected (Hoffmann and Sgrò 2011; Bush et al., 2016).

Previous research has shown that rapid adaptation can occur across a wide range of taxa (Hendry et al., 2008; Whitney & Gabler, 2008). For example, rapid adaptation has been documented in phenology in flowering plants (Franks et al., 2007; Franks et al., 2016), in range expansion in invasive plants (Whitney & Gabler, 2008; Colautti & Barrett, 2013; Zenni et al., 2014), in life history and thermal tolerance traits in insects (Geerts et al., 2015; Tejeda et al., 2016), and in physiological traits in alpine chipmunks (Bi et al., 2019). Furthermore, a review examining over 68 different systems found that rates of phenotypic change occurred more rapidly when induced by anthropogenic change than within natural systems (e.g., Hendry et al., 2008). Importantly, this indicates that many species have the ability to adapt in a time-frame consistent with global warming. However, the factors that contribute to the potential for rapid adaptation are still not fully understood (Hoffmann and Sgrò 2011; Osmond & de Mazancourt, 2012). Rapid adaptation may be complicated by genetic covariances; where selection on one trait may constrain or promote indirect selection on another trait. For example, genetic covariances between stress-resistance traits in Australian *Drosophila* species were found to potentially promote adaptation to climate extremes because

indirect selection aligned with the patterns in genetic variances (Hangartner et al., 2019). However, this could constrain evolution if multivariate genetic variation was found to oppose the direction of selection (Blows & Hoffmann, 2005).

Rapid adaptation to climate change can be assessed in multiple ways, but examining the effects of changing environmental temperatures is the most relevant way to predict the outcome of climate change because a large component of climate change is associated with global warming (Huey et al., 2012; IPCC 2019). Environmental temperature is one of the most important abiotic factors determining why a species lives where it does (Overgaard et al., 2014). This is especially true for ectotherms, which include almost all animals other than mammals and birds (hence encompassing > 98% of the world's animal species). In ectotherms, environmental temperature directly controls body temperature because they have limited ability to physiologically thermoregulate and have a low-thermal inertia (Stevenson, 1985; Cossins & Bowler, 1987; Angilletta, 2009; Hoffmann, 2010; Overgaard et al., 2014). Consequently, environmental temperature influences most physiological and behavioural properties in ectotherms through its influence on body temperature (Huey & Stevenson, 1979; Huey, 1982; Angilletta et al., 2002).

In the absence of biotic interactions, temperature-dependent physiological and behavioural traits, such as thermal performance, thermal tolerance, and thermoregulatory behaviour, shape the thermal space a species inhabits (Svanback & Bolnick, 2007; Gvoždík, 2018). These traits interact to determine a species' thermal niche. The thermal niche is often defined as the thermal space where population growth is greater than zero (Gvoždík, 2018). As environments increasingly change as a result of human impacts, the current thermal space available for a species will also change. The properties of a species' current thermal niche (i.e., thermal performance, tolerances, and behaviour) will dictate whether they can adapt to the changing thermal space.

Even more concerning for species than gradual warming is that climate change is predicted to increase the frequency, intensity, and duration of extreme heat events (Easterling et al., 2000; Meehl & Tebaldi, 2004). Models predict that 20-year heat events will now occur every two years over the next century (Collins et al., 2013). Extreme heat events, termed 'heatwaves', are generally defined as periods lasting longer than three days where observed temperatures exceed the maximum average temperatures by 5°C or more (Vinagre et al., 2018; WMO 2018). Heatwaves challenge an individual's thermal tolerances through their immediate response to acute heat, disregarding whether they have the ability to evolve rapid adaptations to climate change more generally (Reusch et al., 2005). Responses to extreme

heat events are as important (if not more important; see Soroye et al., 2020) as longer-term adaptations to species survival under climate change (Grant et al., 2017; Harris et al., 2018).

Impacts of heatwaves include changes to life history traits such as growth rate (e.g., (Paaijmans et al., 2013; Van Dievel et al., 2017) and germination (e.g., Guerrero-Meseguer et al., 2017); changes to thermal fitness (e.g., Clusella-Trullas et al., 2011; Paaijmans et al., 2013; Van Dievel et al., 2017); decreased population health (e.g., Mouthon & Daufresne, 2006; Kingsolver et al., 2013; Teskey et al., 2015), sudden mass mortalities (e.g., Allison, 2004; Miriti et al., 2007; Garrabou et al., 2009) and decreased genetic diversity (Reusch et al., 2005; Coleman et al., 2020; Gurgel et al., 2020) that can cause inbreeding (Coleman et al., 2020; Gurgel et al., 2020). Disruptions to community structure are also evident (e.g., (Jöhnk et al., 2008; Sorte et al., 2010) through food web changes (e.g., Carreira et al., 2017; and reviewed in Parmesan et al., 2000). Heatwaves may even act as the primary driver of evolution on thermal tolerances (Hoffmann, 2010; Denny & Dowd, 2012; Kingsolver et al., 2013; Buckley & Huey, 2016).

Clearly, heatwaves can have broad implications on ecological communities and, as such, sudden and severe temperature rise needs to be considered at least equally to gradual temperature rise when investigating adaptation under climate change. Here, I aim to examine both of these forces by looking at how they affect the main determinants of a species thermal niche: thermal performance, thermoregulatory behaviour, and thermal tolerance.

1.2. Thermal performance

Thermal performance refers to how fitness-related traits change across a range of relevant environmental temperatures (MacLean et al., 2019). Thermal performance can be measured on traits directly related to fitness (e.g., fecundity, survival, reproductive output) or traits that are proximate measures of fitness (e.g., locomotor speed, morphological trait size, growth rate; MacLean et al., 2019). The ability of a species to rapidly adapt to climate change will be determined by the amount of additive genetic variation in a fitness-related trait that a population has, since additive genetic variation is necessary for species to adapt to new environments (Fisher, 1930). However, the amount of genetic variation in a trait can change in different environmental conditions (Falconer & Mackay, 1996; Hoffmann & Schiffer, 1998; Sgrò & Hoffmann, 1998a; Sgrò & Hoffmann, 1998b; Hoffmann & Merilä, 1999).

Climate change is not only predicted to gradually change current conditions but also increase environmental variability (Thornton et al., 2014), both of which will directly alter

the amount of genetic variation and directly affect the rate of evolution of a trait. Hence this is important to consider when determining the evolutionary potential of a trait in the context of a changing climate. This is because a heritability estimate that tells us the potential of a genetically-based trait to be passed on from parent to offspring can only accurately be applied in the context of the environment in which the estimate was measured (Falconer & Mackay, 1996; Bubliy & Loeschcke, 2000).

Much research has been conducted into how heritability and genetic variance changes under varying environments, with considerable focus on novel and stressful conditions. However, little consensus has been reached on overall or consistent patterns. Some studies show heritability can decrease in stressful environments (e.g., Blum, 1988; Kristensen et al., 2015) due to environmental variance increasing while other variance components remain the same (for example, see Hoffmann & Schiffer, 1998). Other studies show heritability increasing in stressful conditions due to an increase in additive genetic variation caused by exposure to novel environments (e.g., Sgrò & Hoffmann, 1998a; Swindell & Bouzat, 2006).

The inconsistency in patterns has created challenges for predicting evolutionary potential to climate change. This is particularly relevant for ectotherms living close to their upper thermal limits because they are expected to experience increasing temperature stress as the climate warms (van Heerwaarden et al., 2015). It is therefore important that genetic variance and heritability values are specific to the species and environment of interest. However, determining this for a specific trait within a population or study species is often difficult due to time and logistical constraints. **A major focus of this thesis was to investigate how genetic variance and heritability of fitness traits change from a benign to a stressful thermal environment to investigate whether a consistent pattern exists** (Chapter 2).

Another method that may prove to be reliable and quicker in estimating genetic variances across environments is to identify a proxy measure—i.e., using a known genetic and phenotypic correlation with a direct fitness trait. Many experiments and studies would benefit if a proxy measure was identified that is both more convenient to measure and has a high genetic and phenotypic correlation to the direct fitness trait in question when assessed under similar environmental conditions. **I aimed to investigate whether phenotypic and genetic correlations exist between an important fitness trait and a more-easily obtained morphological trait, and whether there is a consistent pattern to these correlations across thermal environments** (Chapter 2).

Aside from quantitative genetic experiments that measure the amount of genetic variance in fitness across environments, the most common way to measure thermal performance is to quantify it as a thermal performance curve. Thermal performance curves (TPC) measure changes in fitness across environmental temperatures (Angilletta, 2009). The information described by TPCs is fundamental to theoretically and conceptually understanding thermal adaptation (Huey & Kingsolver, 1989; Gilchrist, 1995; Angilletta, 2009) and is relevant to climate change researchers looking to relate predictive models to current fitness (Angilletta, 2009; Huey et al., 2012; Kellermann et al., 2019). **Here, I used both quantitative genetic designs** (Chapter 2) **and TPCs** (Chapters 4 and 5) **to examine how thermal performance of key fitness traits change as a result of sudden and gradual temperature rise. An additional objective was to create equipment that can easily be used across the ecology and evolutionary biology field to test TPCs and investigate diverse questions related to the thermal niche** (Chapter 3).

Key fitness traits

Direct measures of fitness, known as 'ultimate' or 'Darwinian' fitness traits, quantify an individual's relative lifetime reproductive success. In many ectotherms, 'fecundity' is a commonly used measurement of ultimate fitness because it quantifies the number of eggs a female lays over a certain time period. The number of offspring that hatch and survive to adulthood is an additional ultimate fitness measure and is referred to as 'productivity'. 'Development rate', which measures the time the egg was laid to the time it develops into an adult, is also an ultimate fitness trait because relatively quicker development times produce a quicker generation turn-around-time (where more generations in the same time period results in more progeny and therefore potentially higher relative fitness). Quicker development rates also aid offspring by helping them outcompete other individuals by having resources available to them before others hatch (Chippindale et al., 1997).

Indirect measures of fitness are usually quantified by measuring other life history or morphology traits. For example, body size is considered an important fitness trait because it is related to fecundity, with larger bodied individuals shown to exhibit a higher fecundity (e.g., Chiang & Hodson, 1950; Santos et al., 1992). Many studies assume that wing size in winged insects correlates to body size and therefore can be used as a proxy (Bullock, 1999; Loeschcke et al., 1999; Outomuro & Johansson, 2011; Yeap et al., 2013; Dellicour et al., 2017). **In this thesis, I assessed thermal performance by examining genetic variances**

and correlations of fecundity and wing size (Chapter 2), **and measured TPCs for productivity, development rate, and wing size** (Chapter 4 and 5) **of two *Drosophila* species**.

1.3 Thermoregulatory behaviour

Thermoregulatory behaviour is particularly important for ectotherms because they have a very limited capacity to physiologically thermoregulate (Stevenson, 1985) and otherwise, would be extremely vulnerable to environmental conditions. To overcome this, ectotherms use behaviour as a means to regulate body temperature (Cowles & Bogert, 1944). For ectotherms, behavioural thermoregulation is controlled by temperature preference (Angilletta, 2009). Behaviour can be used to thermoregulate by helping animals avoid dangerous temperatures (Norris, 1967; Grant & Dunham, 1988; Dillon et al., 2009), or by promoting exposure to physiologically ideal temperatures (Huey et al., 2003). In both of these instances, behavioural thermoregulation (and hence temperature preference) can be adaptive (Dillon et al., 2009).

Consequently, temperature preferences are thought to closely match the optimal physiological temperature in many species (Huey & Bennett, 1987; Huey & Kingsolver, 1989). This is known as the 'thermal coadaptation hypothesis'. The thermal coadaptation hypothesis can be used to discern information on the relationship between thermoregulatory behaviour and thermal performance, which can aid researchers in determining how behaviour may influence adaptation to climate change. For example, behaviour may limit adaptation to climate change because animals may avoid changing temperatures by moving to less variable microhabitats. Conversely, if behaviour promotes exposure to the changing conditions (e.g., if temperature preferences are higher than the current average environmental temperature), than thermoregulatory behaviour may aid rapid adaptation in a warming-climate scenario.

Research on the thermal coadaptation hypothesis has mainly focused on large ectotherms and has used indirect measures of fitness (for reviews see Angilletta et al., 2002; Halliday & Blouin-Demers, 2015). Previously, only three studies have investigated how optimal temperatures for *ultimate* fitness traits relate to temperature preferences (Anderson et al., 2011; Halliday & Blouin-Demers, 2015; Halliday & Blouin-Demers, 2017). However, a major gap in this research is that none of these studies incorporated oviposition temperature preference into behavioural measurements. This is key because natural selection will favour females that avoid ovipositing in lethal thermal environments and prefer ovipositing in

optimal thermal environments (Jaenike, 1978; Thompson, 1988; Mery & Kawecki, 2004; Gripenberg et al., 2010; Soto et al., 2011). Incorporating oviposition preference site into temperature preference measurements allows for a more complete measurement of thermoregulatory behaviour because oviposition temperature preference is thought to correspond to adult temperature preference (Dillon et al., 2009).

In addition, direct comparisons on the thermal coadaptation hypothesis between generalists and specialists are lacking. Because generalists and specialists have evolved different thermal performance strategies—where generalists perform within a broader range of thermal environments than specialists, but specialists exhibit a relatively-higher performance capability than generalists within that narrower range—it can be deduced from the thermal coadaptation hypothesis that their thermal preferences should reflect this. Here, I measured TPCs for ultimate fitness traits in a generalist and specialist species of *Drosophila* and compared them to temperature preference that incorporated oviposition preference site. This will supplement the currently lacking literature for the thermal coadaptation hypothesis in terms of ultimate fitness traits and in investigating the evolutionary differences of the thermal coadaptation hypothesis in a generalist versus specialist species. In doing so, **I aimed to investigate whether temperature preference is co-adapted to thermal performance of productivity, development rate, and wing size in both a thermal generalist and a thermal specialist species** (Chapter 4).

1.4 Thermal tolerances

Thermal tolerances refer to the capacity of an animal to survive short-term exposure to extreme temperatures (MacLean et al., 2019), and are an important limiting factor to an ectotherm's distribution (Cossins & Bowler, 1987; Angilletta, 2009; Sunday et al., 2011). Information on thermal tolerance limits can provide key information on how temperature may restrict an ectotherm's current and predicted distribution under climate change (van Heerwaarden & Sgrò, 2013; Seebacher et al., 2015). This is especially important when considering that climate change will increase extreme temperature events. Extreme temperature events may promote adaptation of thermal tolerances (Denny & Dowd, 2012; Buckley & Huey, 2016) to help species adapt alongside climate change.

Small ectotherms (such as *Drosophila)* are often used as model species to investigate the adaptive potential of thermal tolerance (Angilletta et al., 2002; Hoffmann et al., 2003b; MacLean et al., 2019). In terms of climate change, it is important to estimate thermal

tolerance parameters that are relevant to rising temperatures and heat tolerance. In *Drosophila*, some studies have found limited evolutionary potential for traits associated with heat tolerance (e.g., Mitchell & Hoffmann, 2010; Sunday et al., 2011; Kellermann et al., 2012; Araujo et al., 2013; Hoffmann et al., 2013; Castañeda et al., 2019), while others have found high adaptive potential for upper thermal limits (e.g., Bubliy & Loeschcke, 2005; Folk et al., 2007; Blackburn et al., 2014; Geerts et al., 2015; van Heerwaarden et al., 2015). Research has also shown that upper thermal limits in *Drosophila* have limited plasticity (Overgaard et al., 2011), indicating that many species may be vulnerable to increasing temperatures if they are not able to adapt.

Previous research on thermal tolerance adaptation is vast. The majority of previous studies select for thermal tolerances *generation–after–generation* to determine whether tolerances can adapt. However, a gap in the literature remains when looking at thermal tolerance selection in the framework of sudden extreme temperature events. Specifically, there has been no previous empirical research investigating how selection caused by a heatwave affects the heat tolerance of a population *generations later with no further selection*. This is necessary knowledge because heatwaves often occur during heatwave 'seasons' (i.e., annually), meaning that there is often a large generation time-lag between one heatwave selection event and the next, especially for organisms with rapid generation times. **A major focus of this thesis was to investigate whether one heatwave, that selects for a high thermal tolerance in a single generation, affects the long-term thermal tolerance of that population (i.e., after multiple generations with no further selection in between**; Chapter 5). I aimed to determine whether selection on thermal tolerances of a single-generation can aid subsequent generations in surviving a second heatwave. It's theorized that heatwaves will cause directional selection for heat tolerant phenotypes, and this, in turn, may increase resilience towards future heat events. In this aspect, heatwaves may aid species by causing rapid adaptation to increasing temperatures and increasing temperature variability—recently termed a potential 'silver lining' of a heatwave (Coleman & Wernberg, 2020).

I also investigated how one heatwave affects the long-term thermal performance of the population after multiple generations with no further selection (Chapter 5). It has been increasingly shown that temperature extremes can have a large impact on a population's phenotype composition, possibly larger than gradual temperatures rise (Kingsolver et al., 2009; Kjærsgaard et al., 2010; Rego et al., 2010). Therefore, how sudden temperature rise affects long-term thermal tolerances, as well as thermal performance, will aid in understanding the effects of climate change on a species thermal niche.

1.5 Study system

I used two sister-species of *Drosophila* as the study-system in this thesis. *Drosophila* are frequently used as model species to understand genetic and phenotypic variances of fitness traits (Hoffmann, 2009) and adaptive potential of thermal performance and thermal tolerances (MacLean et al., 2019). This is because *Drosophila* are a widely diverse genus with over 2000 species that live in a wide variety of natural environments, are dependent upon multifaceted ecosystems, and live in complex communities with competitors, parasitoids, and predators. Hence, they are pertinent species for ecology and evolution studies and are highly amendable to experimental manipulation and intergeneration measurements because of their short generation time and relatively easy ability to maintain in the laboratory. It is also relevant that the majority of animal species are invertebrates and the majority of vertebrate species are ectotherms, making *Drosophila* an appropriate study species for use in climate change studies.

The two sister-species used in this thesis were *Drosophila birchii* and *Drosophila serrata. Drosophila birchii* and *D. serrata* are closely related species that belong to the *montium* subgroup and are found along the east coast of Australia. *Drosophila birchii* is considered a rainforest specialist and is found within the Australian Wet Tropic region at mid and high elevation (Kelemen & Moritz, 1999; Fig. 1.1). *Drosophila serrata* is considered a generalist species that resides within lowland sclerophyll woodland up into mid and high elevation rainforest that creates a more continuous distribution than *D. birchii* (Schiffer et al., 2004; Fig. 1.1). The pair form a well-studied species complex that are frequently used to investigate climatic adaptations and mating behaviours due to their partial distributional overlap, differences in physiology and habitat choice, and the fact that they are reproductively isolated (e.g., Ayala, 1966; Jenkins & Hoffmann, 1999; Kelemen & Moritz, 1999; Schiffer et al., 2004; Higgie & Blows, 2008). In this thesis, I studied two or more populations of each species. Evolved differences among populations within a species can potentially tell us whether local adaptation has occurred or if a pattern is conserved among different populations (Catullo et al., 2019).

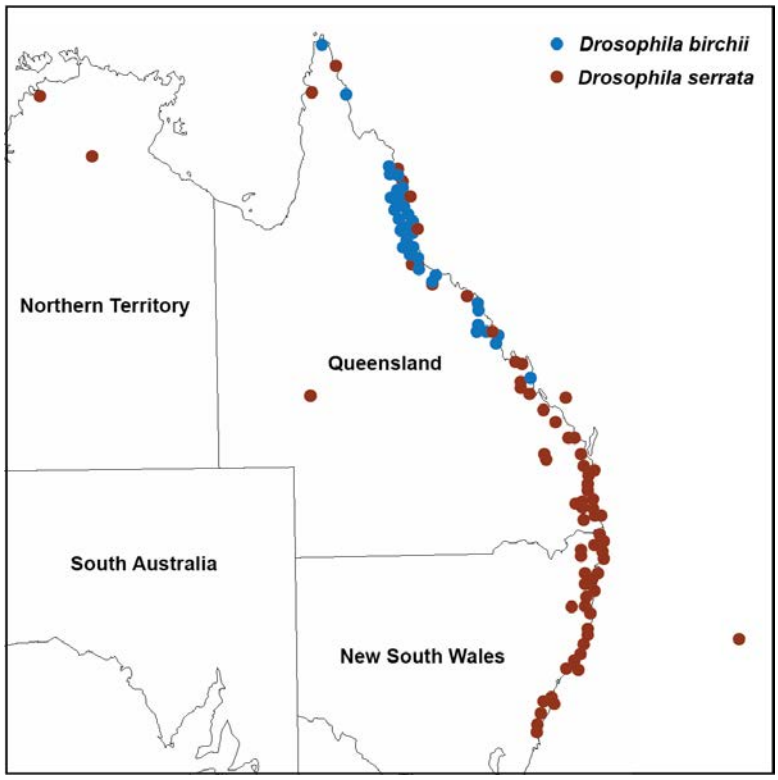

Figure 1.1: Distribution of *Drosophila birchii* and *Drosophila serrata* in Australia.
Drosophila birchii is a specialist species of fruit fly confined to mountain-top rainforests within the Wet Tropics Region of Australia (blue), and *D. serrata* is a generalist species that can be found from Queensland down to the southern part of New South Wales, Australia (red). Figure adapted from the Australian Drosophila Ecology and Evolution Resource (ADEER; Hoffmann et al., 2015b) and datasets and distributions obtained from Hallas et al. (2002), Hoffmann & Shirriffs (2002), Griffiths et al. (2005), Schiffer & Mcevey (2006), Hoffmann et al. (2015a).

1.6 Aim of thesis

Understanding the ability of a species to rapidly adapt to environmental temperature is essential for predicting the potential impacts of climate change on ecological communities (Guisan & Thuiller, 2005). Here, I aimed to investigate the impacts of gradual global warming and extreme temperature events on key components of a species thermal niche by assessing the adaptive potential of thermal performance and thermal tolerance, and examining how thermal behaviour correlates to thermal performance. Specifically, I:

- Studied the effects of thermal stress on genetic variance of key fitness and morphological traits in a generalist and a specialist (Chapter 2).
- Studied the effects of thermal stress on phenotypic and genetic correlations of key fitness and morphological traits in a generalist and a specialist (Chapter 2).

- Designed adjustable and customisable temperature arrays for controlled experiments to investigate thermal performance, thermal behaviour, and thermal tolerance (Chapter 3).
- Studied whether thermal behaviour and thermal performance are coadapted for key fitness traits and examined if this relationship is maintained in both a generalist and a specialist (Chapter 4).
- Studied how one sudden extreme heat event (i.e., heatwave) affects the ability of a specialist to withstand a second heatwave by examining effects on the long-term thermal tolerance and long-term thermal performance of key fitness traits (Chapter 5).

1.7 Structure of thesis

The main data chapters in my thesis are presented as four stand-alone, but interrelated, manuscripts (Chapters 2–5; Fig. 1.2) that have been published (Chapter 3), have been submitted for publication (Chapter 2) or will be submitted for publication (Chapters 4 and 5). I have taken care to avoid repetition whenever possible, but this format has caused some areas of unavoidable repetition in the introduction and method sections. I have changed plural pronouns throughout to a singular pronoun for the purpose of this thesis, but it is important to note that all manuscripts include multiple co-authors. The contributions of co-authors can be found at the beginning of this thesis and at the beginning of each chapter. Supplementary information and material are located at the end of the thesis in appendices to facilitate reading of the main text.

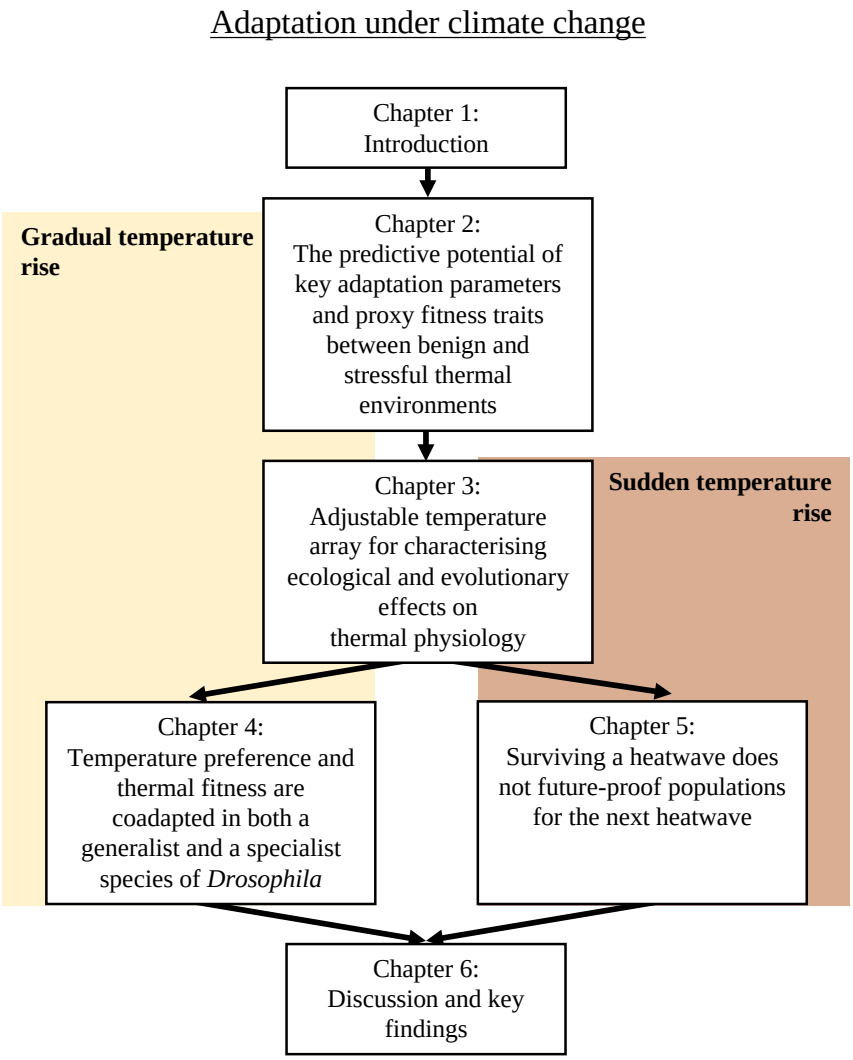

Figure 1.2: Flowchart of thesis.

This flowchart outlines the chapters of this thesis and shows how each chapter fits within the main goal of investigating how important components of a species thermal niche is affected by gradual and sudden temperature change.

Chapter 2: The predictive potential of key adaptation parameters and proxy fitness traits between benign and stressful thermal environments

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This chapter is under review at the journal *Heredity*.

Contributions: JC and EO conceived the experiment, with important input from MH and CH. JC conducted the experiment. JC and HS collected data. JC analysed data with important input from EO and MH. JC wrote the manuscript with important contributions from EO, MH, and CH.

2.1 Abstract

Understanding the adaptive potential of a species is important when trying to predict whether the species can contend with climate change. Adaptive capacity depends on the amount of genetic variation within a population for a particular trait; but genetic variation changes in different environments making it hard to predict whether a trait can adapt. Understanding how genetic variation changes across environments is therefore critical to predicting adaptive potential. A trait that is strongly correlated with fitness across environments may provide a proxy measure to aid in understanding adaptation potential. Here, I investigated how genetic variances and phenotypic and genetic covariances between a fitness trait and two morphological traits changed between thermal environments in two closely-related *Drosophila*. I used a parent-offspring quantitative genetic design to test the effect of a benign (23°C) and stressful (28°C) thermal environment on genetic variances of fecundity and wing size and shape, as well as their phenotypic and genetic covariances. Overall, I found genetic variances were higher within the stressful environment for fecundity but lower within the stressful environment for wing size. I did not find evidence for significant phenotypic correlations. Phenotypic and genetic correlations did not reveal a consistent pattern between thermal environments *or* between species, populations, or generations. This corroborates previous research that was unable to find a trend in how environments affect genetic variance. This is important because conclusions drawn in one environment about the adaptive potential of a trait, and the relationship of that trait with fitness, cannot be extrapolated to other environments *or* to closely-related species, populations, or across generations. This confirms that researchers need to use caution when generalising findings across environments in terms of genetic variation and adaptation potential.

2.2 Introduction

Climate change is causing increased temperatures that will impose stress on species (Thomas et al., 2004). Many species lack the ability to disperse to more optimal environments (Bellard et al., 2012; Ceballos et al., 2017), and will have to adapt to the stressful temperatures to survive in the long-term (Thomas et al., 2004; Hoffmann & Sgrò, 2011). Adaptation potential will depend on the amount of genetic variation in traits relevant to the selection imposed by environmental change (Fisher, 1930; Falconer & Mackay, 1996), and adaptation will need to be rapid given the speed of human-induced climate change. Understanding the adaptive potential of species, especially those currently living close to their upper thermal limits, is therefore crucial in today's changing climate (Urban et al., 2016; Funk et al., 2019; Shaw, 2019).

Importantly, genetic variation is context-dependent—meaning the amount of genetic variation in a trait in a given population can change under different environments (Falconer & Mackay, 1996; Hoffmann & Schiffer, 1998; Sgrò & Hoffmann, 1998a; Sgrò & Hoffmann, 1998b; Hoffmann & Merilä, 1999). Short-term environmental changes can play an important role in adaptive evolution (Wood & Brodie, 2016) and can induce a similar or larger change in genetic variance than changes to the genetic architecture that accumulate over hundreds of generations between populations (for review, see Wood & Brodie, 2015). Increases in environmental variability, such as those predicted with climate change, will therefore directly affect the rate of evolution of a trait—as environments get warmer, not only may the type of selective pressure change, but also the potential for the trait to respond to selection. This is important because as researchers aim to determine whether species can adapt to climate change, the changing climate itself may increase or decrease adaptation potential.

Much research has focused on examining whether there is a consistent pattern to changes in expression of genetic variance (for reviews, see Sgró & Hoffmann, 2004; Rowinski & Rogell, 2017; and Fischer et al., 2020). However, there is no consensus on whether stressful conditions increase or decrease the expression of genetic variance. The majority of studies focus on quantifying genetic variance by calculating heritability (h^2), which describes the proportion of genetic variance due to additive effects. This can be used to predict the magnitude of the response to selection via the breeder's equation. These studies show both increases (e.g., Sgrò & Hoffmann, 1998a; Sgrò & Hoffmann, 1998b; Swindell & Bouzat, 2006) and decreases (e.g., Hoffmann & Schiffer, 1998; Bubliy et al., 2001; Kristensen et al., 2015) in heritability under stressful conditions. Increased heritability may

result from novel genetic variance that is expressed when exposed to new conditions (i.e., 'cryptic genetic variance'; see review by Hoffmann & Schiffer, 1998; but also see Swindell & Bouzat, 2006). Decreased heritability may result from low cross-environment genetic covariances (Fischer et al., 2020), or environmental variance increasing while other variance components remain the same (for example see Hoffmann & Schiffer, 1998). Recently, studies have recommended quantifying genetic variance using parameters standardized by the trait mean—such as coefficient of additive variance (CV_A) and its square, evolvability (I_A)—because estimates of heritability can be influenced by sources of non-genetic environmental variation that may preclude comparison across environments and traits (Houle, 1992; Rowiński & Rogell, 2017; Fischer et al., 2020). Parameters standardized by the trait mean may also more intuitively indicate whether trait shifts will be substantial because the magnitude of change is more easily understood.

Assessing the effect of a changing environment on genetic variance is further complicated when attempting to measure genetic variance across different environments for fitness. Direct fitness (reproductive success) is often difficult to measure in the wild because of uncontrolled and unmeasured factors (Orr, 2009), and in the laboratory due to time and logistical constraints (Rosenberg, 1982; Nguyen & Moehring, 2015). Instead, a morphological trait that strongly correlates with fitness, and is more easily measured, may provide a good proxy when fitness measures are difficult to obtain. If phenotypic correlations between a morphological trait and fitness are strong, researchers can use the easier-to-measure trait to predict genetic variation of fitness across different environments (Arnold, 1983).

More importantly, a strong phenotypic correlation may indicate that two traits are genetically linked through physical linkage, pleiotropy, or linkage disequilibrium (Cheverud, 1988; Conner & Via, 1992; Roff, 1995; Blows & Hoffmann, 2005). This is important because a positive genetic correlation between traits could aid adaptation to novel environments if selection favours that trait combination by augmenting the effect of selection on the correlated fitness trait (Blows & Hoffmann, 2005; Agrawal & Stinchcombe, 2009; Walsh & Blows, 2009; Holman & Jacomb, 2017). Therefore, determining genetic correlations of traits with fitness is an important part of the puzzle when predicting evolutionary potential.

However, much like genetic variation in individual traits, phenotypic and genetic covariances between traits (or between a trait and fitness) can vary depending upon the environment in which they are measured (Sgrò & Hoffmann, 2004)—meaning measurements

obtained in one environment cannot necessarily be generalised to other environments. For example, adult female body mass and her egg size were positively correlated on one host-plant species and negatively correlated on a different host-plant species in a beetle (Czesak & Fox, 2003). Genetic correlations can change within novel environments due to genotype-environment interactions—where genes that affect a trait in one environment may not be influential in a different environment (Sgrò & Hoffmann, 2004). In some instances, the loci that contribute to covariances through pleiotropy or physical linkage have specifically been found to be influenced by environmental effects (e.g., Gutteling et al., 2007; Hausmann et al., 2005). However, more empirical data are needed to understand whether there are patterns to how genetic variances and covariances of morphological and fitness traits vary across thermal environments (Rowiński & Rogell, 2017; Fischer et al., 2020).

Drosophila are often used to investigate genetic variances and covariances across environments due to their short generation time and ability to produce large numbers of offspring that allow for quantitative genetic experimental designs. Fecundity is a commonly assessed fitness trait in *Drosophila*. However, measuring fecundity can often prove time- and labour-intensive and logistically challenging. Ecological theory assumes that body size is correlated with fecundity, with larger individuals exhibiting a higher fecundity (Chiang & Hodson, 1950; Santos et al., 1992; Robertson, 1956), and wing length has been shown to phenotypically correlate with fecundity (Tantawy & Vetukhiv, 1960; Woods et al., 2002). However, two key studies examining the relationship of wing length and fecundity in *Drosophila* when exposed to stressful environments found mixed evidence. Sgrò & Hoffmann (1998b) did not detect a significant positive phenotypic or genetic correlation in a cold-stress, heat-stress, or benign environment. They also did not find a significant genetic cross-environment correlation (parents raised in one environment and offspring raised in a different environment) between cold-stress, heat-stress, or benign environments (Sgrò & Hoffmann, 1998b)—meaning that they did not find a correlation between wing length and fecundity among and between any experimental environment. Conversely, Woods et al. (2002) found significant positive phenotypic correlations (for two of three generations) and significant positive genetic correlations between wing length and fecundity in a stressful environment, but not in a benign environment.

With advances in technology over the past decade (i.e., advances in microscopic imaging and digitizing), more intricate morphological traits such as wing size and wing shape have been increasingly used in place of wing length. However, very few studies have examined genetic variation and heritability in wing size and shape (Gilchrist & Partridge,

1999; Hoffmann & Shirriffs, 2002; Moraes et al., 2004); and, to my knowledge, only one has examined the phenotypic and genetic correlations of wing size with fecundity (Woods et al., 2002). Wing size and wing shape in *Drosophila* have a polygenic basis independent of one another (Carreira 2011), so phenotypic and genetic correlations of each of these traits with fecundity may differ. Wing size exhibits a history of directional selection in *Drosophila*, whereas wing shape has been shown to undergo optimizing selection (Gilchrist & Partridge, 2001). Although most of the fundamental research uses wing length as a trait that is highly correlated to thorax size (and therefore body size; Chiang & Hodson, 1950; Tantawy & Vetukhiv, 1960; Santos et al., 1992; Woods et al., 2002), wing size may be a better indicator of overall body size because it is a product of more complex interactions between the different wing compartments (i.e., anterior and posterior compartments; Guerra et al., 1997; Gilchrist & Partridge, 1999). Hence, wing size may account for a greater proportion of variation than wing length alone. Wing shape is important for flight performance in *Drosophila* and has been shown to exhibit high heritability (Hoffmann & Shirriffs, 2002; Moraes et al., 2004).

Temperature as a stressor is contextually important in today's climate, but it has only been used in one *Drosophila* study to assess whether genetic correlations exist between fecundity and wing length, and whether heritability changes between different thermal regimes (i.e., *D. melanogaster*; Sgrò & Hoffmann, 1998b with the same data used in Woods et al., 2002). Here, I focused on whether genetic variances in fecundity change across thermal environments, and whether a morphological trait that may be a good proxy of fitness in one environment was also a good proxy in a stressful thermal environment. I examined the consistency of heritability, coefficient of additive genetic variance, and evolvability between thermal environments (one benign and one stressful), generations, and within and between two sibling species of *Drosophila*. A strength of this study is that I assessed both life history and morphology traits in two closely-related species to see whether this pattern was conserved. I also assessed the phenotypic and genetic covariances of these traits. The correlation of body morphology with fitness informs us about the strength and direction of selection. This is important because patterns of selection in one environment may not reflect similar responses in another environment.

2.3 Methods

Experimental populations

Two sibling species of fruit fly found along the east coast of Australia were used in this study: *Drosophila serrata*, a generalist species found in forested areas; and *D. birchii*, a specialist species confined to tropical rainforest ecosystems (Schiffer & Mcevey, 2006; Higgie & Blows, 2008). Mass bred populations from two different geographical areas for each species were used. Each mass bred population was originally created by breeding the offspring of ten isofemale lines collected from field sites within Queensland, Australia. *Drosophila birchii* flies were collected from Paluma National Park (19° 0'16.27"S, 146°12'35.59"E) and Mt. Lewis National Park (16°35'30.36"S, 145°16'27.78"E). *Drosophila serrata* flies were collected from Paluma National Park (19° 0'16.27"S, 146°12'35.59"E) and Raglan Creek (23°42'49.74"S, 150°49'0.10"E). All flies were collected between February and May 2016. Isofemale lines were maintained in controlled laboratory conditions for 18 generations before mass bred populations were created. All isofemale lines and stocks were maintained at large population sizes (isofemale lines: $N > 500$, stocks: $N > 1000$) to retain natural genetic variation. Flies were reared on standard *Drosophila* food that contained sugar, yeast, and agar as described in Higgie and Blows (2008). All flies were reared under constantly controlled laboratory conditions of 23°C ± 1°C, 50% relative humidity (RH), and 12 hr light:dark cycles.

Quantitative genetic experimental design

A parent-offspring breeding design was used to assess heritability and phenotypic and genetic covariances of fecundity and wing morphology at a benign (23°C) and a stressful (28°C) temperature (Fig. 2.1). The benign temperature (23°C) represents an approximate average temperature each species experiences across their range both temporally and spatially, as well as the optimal rearing temperature in the laboratory. A temperature of 28°C was chosen as a stressful thermal environment as it was found to be within the upper margin of the thermal niche for *D. birchii* and to place stress upon *D. serrata* (from pilot studies showing reduced survival). However, it is important to note that the level of stress placed upon each species may differ due to their different physiologies—meaning comparisons can only be made within species. Full development was expected in both species based on previous research.

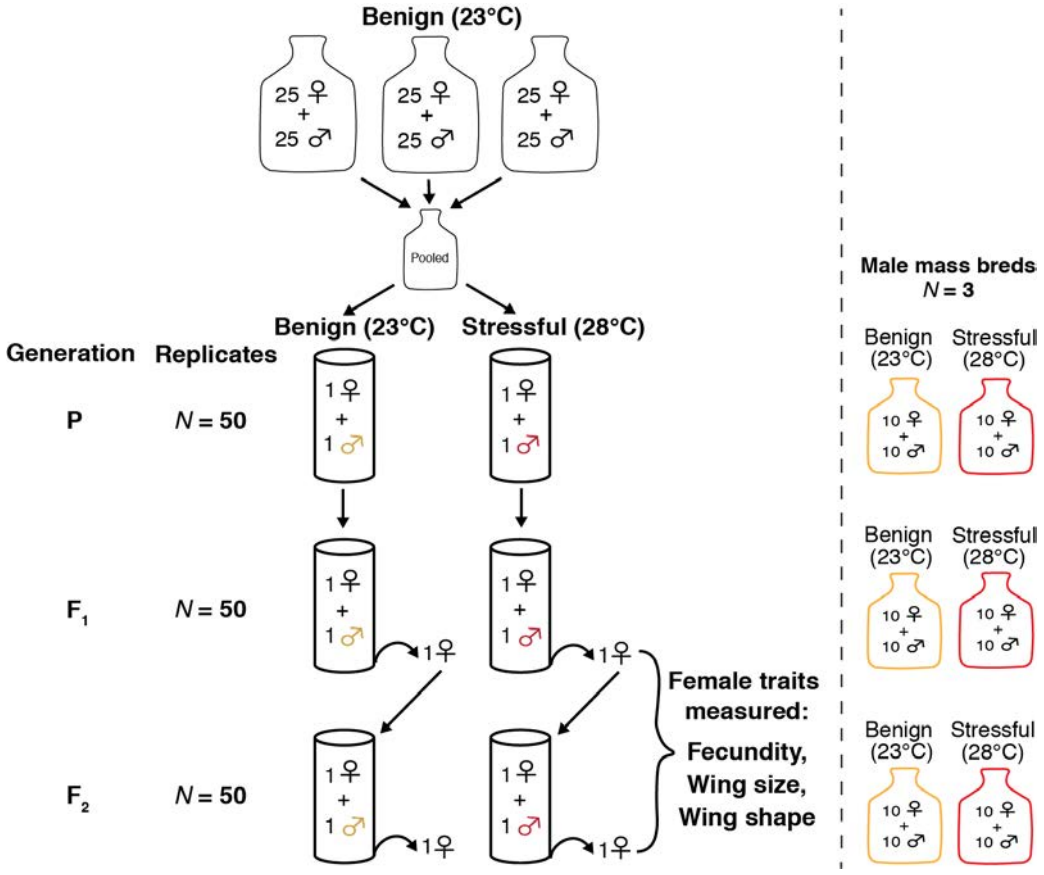

Figure 2.1: Parent-offspring quantitative genetic experimental design.

The parent-offspring quantitative genetic experimental design used to measure female fecundity, wing size, and wing shape on both dams and daughters. This design was used for two populations of both *D. birchii* and *D. serrata*. Experimental female flies were raised in either a non-stressful rearing temperature (23°C) or a stressful temperature (28°C). Mass bred populations were raised alongside each generation and supplied males for mating purposes.

Two generations before the start of the experiment, density-controlled mass bred populations were created for each species and population by sexing 25 females and 25 males from the laboratory stock and placing them in one 300 mL bottle with 100 mL of food. This was repeated three times for each species and population. Flies were removed from each replicate bottle after 72 hrs and bottles were carded for pupation. Offspring were collected at random and sexed to subsequently create family lines and stock mass bred populations for each species and treatment.

One generation before the start of the experiment (i.e., P generation; Fig. 2.1), virgin offspring were sexed from the density-controlled mass bred populations using CO_2 anesthetization. Flies were placed in 100 mL holding vials with 5 mL of food for 72 hrs to

allow for sexual maturation and full recovery from anesthetization, with 5 individuals per holding vial. After this, one male and one female were randomly collected and placed in a 100 mL glass vial with 10 mL of food, stoppered with a porous stopper, and directly placed in an incubator set to the relevant temperature for each thermal environment treatment. Humidity inside the vials was expected to remain at approximately 90% RH (from preliminary experiments), and a 12 hr light:dark cycle was maintained. This was done for 50 family replicates for each species, population, and treatment. Mating pairs were allowed to mate for 48 hours before being removed from the vial. This ensured all experimental flies were reared in a controlled and low-density environment. In addition, three low-density stock bottles containing 10 females and 10 males were created and maintained for both the parent and offspring generations to provide a supply of males for mating to assess fecundity (i.e., male mass breds; Fig. 2.1). These were maintained in each thermal environment and males were randomly collected from each bottle and mated with a female from the same experimental rearing temperature.

Fecundity measurements

Virgin female offspring of each family replicate vial were sexed under light anesthetization and placed in holding vials for 72 hrs. One female (i.e., dam) from each F_1 family was randomly selected and placed in an empty vial with one virgin male collected from the male stock bottles. Each vial contained a small spoon with 2 mL of food to provide a medium for oviposition. The food was dyed green to aid in counting eggs, and a drop of a live yeast-water solution (1 g baker's yeast:10 mL water) was spread over it to promote ovipositing. Vials were immediately placed within their temperature treatment and flies were allowed to mate for 24 hrs. After 24 hrs, the spoon was removed and immediately frozen at -19°C for eggs to be counted at a later time, and replaced with a new spoon. This was repeated every 24 hrs for three days and a cumulative fecundity count was obtained. Cumulative fecundity measurements from the first three days of maturity are significantly correlated with lifetime reproductive success of female *Drosophila* (Pekkala et al., 2011; Nguyen & Moehring, 2015). After 72 hrs, the mating pair was transferred to a rearing vial with 10 mL of food and allowed to mate for the next 48 hr period before being removed. Females were then immediately frozen for wing size and shape measurements. Daughters of these pairs were

collected from each vial and one virgin female offspring from each mating pair was assessed for fecundity and wing traits using the same methods described above.

Fecundity was scored using a microscope and click counter by counting the number of eggs on each spoon. Approximately one quarter of spoons were counted twice, at random, to assess repeatability; a positive correlation close to 1 indicated that counting was highly repeatable between measurements ($r = 0.994$; $P < 0.001$; $N = 81$).

Wing morphometrics

All dams and daughters were frozen at nine days old to assess wing size and shape. Left wings were removed using fine forceps and mounted on microscope slides with double-sided tape. Wings were photographed using a Leica Image microscope (*LASV3.8*). Images were randomized and collated as a TPS file using *tpsUtil* (Rohlf, 2010b). Landmarks were placed on ten consistent morphometric wing features of each image (Appendix A Figure 1) using the program *tpsDig2* (Rohlf, 2016). Outliers and landmarking errors were identified using *tpsRelW* (Rohlf, 2010a) and corrected or removed before wing measurements were computed.

Landmarked coordinates underwent a Generalised Procrustes Analysis (GPA) superimposition (Rohlf & Slice, 1990), where wing size and alignment are adjusted for by superimposing images upon one another over an average configuration. The GPA superimposition has been found to produce estimates with the least amount of error in a study on geomorphometrics (Rohlf, 2003). The square root of the summed squared distance between centroid configuration and landmarks is known as the centroid size and provides a measure of overall size (Rohlf & Slice, 1990; Rohlf, 2000). Although size effects should be removed via the GPA, a correlation between shape and size might still occur (known as allometry), and hence this was also assessed. In addition to centroid size, the GPA computes a set of Procrustes residuals for each landmark. A principle component analysis (PCA) was conducted upon these to identify variation components, which can be used to describe a single axis of variation in wing shape among individuals (Adams et al., 2004; Zelditch et al., 2004; Gómez et al., 2009). In this instance, the PCA is equivalent to a relative warp analysis because the variation between landmarks was not weighted by bending energy (Zelditch et al., 2004), so PCA scores are equivalent to relative warp (RW) scores. As per common practice, RW scores that explained greater than 5% of variation were used as shape variables

(Zelditch et al., 2004; Gómez et al., 2009) to analyse differences in wing shape using the *geomorph* package in R (Adams et al., 2020).

Analysis

Data was checked for outliers, homogeneity, normality, and independence as outlined in the protocol described in Zuur et al. (2010) and analyses were performed using the statistical program R (R Core Team, 2019). Mean trait values and phenotypic variances (calculated as squared standard errors of the mean trait values) were calculated for fecundity, wing size, and wing shape for both the dam and daughter generations. To test whether thermal environment had an effect on mean trait values and on phenotypic variances, a two-way ANOVA (for thermal environment and generation and its interaction) or generalised least square model was conducted for each trait and metric depending on data structure. Population and its interaction with thermal environment was also included when significant. For the multivariate measure of shape (RW score matrix), a permutational MANOVA (also known as a Procrustes ANOVA; Goodall, 1991) was used to test for differences in wing shape between thermal treatment, populations, and generations. All analyses were conducted separately for each species.

Narrow sense heritability (h^2) for each trait was calculated from a regression of offspring trait values on maternal trait values (Falconer & Mackay, 1996). As I conducted only a single-parent regression, the phenotypic resemblance is equal to half of the genetic variation and thus the slope parameter estimate β represents:

$$\beta = 1/2(\frac{V_A}{V_P})$$

$$\beta = 1/2h^2$$

and so heritability is equal to twice the slope of the regression line (Falconer & Mackay, 1996). In parent-offspring regression, estimates can be greatly skewed if variances found in the parental generation and offspring generation differ (Falconer & Mackay, 1996). To overcome this, I standardized all traits to a mean of zero and standard deviation of one prior to computation of heritability and genetic covariances (Sgrò & Hoffmann, 1998c). The significance of deviations of heritability estimates from zero were assessed using an *F*-test and all *P*-values were adjusted for using the False Discovery Rate method (Benjamini & Hochberg, 1995). Standard errors of heritability were obtained directly from the regression model.

To obtain an overall estimate of heritability for wing shape, I followed the equations set forth in Monterio et al. (2002) for estimating heritability from a parent-offspring regression on a multivariate trait (i.e., wing shape with RW scores > 5% variation included). This was done by first obtaining the coefficient of determination (R^2) from a multivariate linear regression of offspring RW scores onto dam RW scores. The square root of the coefficient of determination (R) was then used in the following formula:

$$\beta = R\frac{S_O}{S_P}$$

where β is the multivariate regression coefficient, S_O is the standard deviation of the offspring trait, and S_P is the standard deviation of the parental trait. The multivariate regression coefficient multiplied by two is then equal to the heritability of the trait (as I still need to account for only having half of the genetic variation due to the single-parent-offspring comparison). The standard error of heritability for the multivariate wing-shape trait was calculated using the number of families (N) and offspring (n; Falconer & Mackay, 1996; Monteiro et al., 2002):

$$s.e(h^2) = \frac{2}{\sqrt{nN}}$$

Significance of deviation from zero for multivariate shape heritability was assessed using a Wilks' lambda test (Zelditch et al., 2004; Gómez et al., 2009).

In addition, coefficients of genetic variation (CV_A) and evolvabilities (I_A) were calculated following Houle (1992) as:

$$CV_A = \frac{\sqrt{V_A}}{\bar{X}}$$

$$I_A = \frac{V_A}{\bar{X}^2}$$

Because I did not directly calculate V_A in my analysis, I obtained estimates based on the method of Garcia-Gonzalez et al. (2012). V_A estimates were calculated by multiplying the total phenotypic variance (V_P) of each trait mean by the narrow-sense heritability (h^2), since $V_A = h^2 \times V_P$ (Falconer & Mackay, 1996). This is an alternative way to calculate CV_A when researchers do not have the sire variance component (V_{sire}) or another direct measure of V_A (Garcia-Gonzalez et al., 2012). Standardized data cannot be used to calculate CV_A and I_A because a scaling correction to a zero mean produces a meaningless comparison and undefined value when dividing by the trait mean a second time (Garcia-Gonzalez et al.,

2012). The above methods were therefore only performed on non-standardized data and CV_A and I_A values were not calculated for RW scores of wing shape as these are standardized.

The phenotypic correlation among each pair of traits was calculated as the Pearson correlation coefficient. Genetic covariances (cov_{XY}) were obtained by regressing one trait in the parental generation onto the other trait in the offspring generation, in both directions, adjusting for relationship, and taking the mean of the adjusted Pearson correlation coefficients as suggested by Falconer and Mackay (Falconer & Mackay, 1996). Genetic correlations were then calculated using the genetic covariances and the following equation:

$$r_G = \frac{cov_{XY}}{\sqrt{cov_{XX} cov_{YY}}}$$

where cov_{XY} is the genetic 'cross-covariance' and cov_{XX} and cov_{YY} are the parent-offspring covariances for the individual traits. Standard errors for genetic correlations were calculated using an approximate formula as proposed by Reeve (1955), Robertson (1959) and explained in Falconer and Mackay (1996):

$$\sigma_{r_G} = \frac{1 - r_G^2}{\sqrt{2}} \sqrt{\left[\frac{\sigma_{(h_X^2)}\, \sigma_{(h_Y^2)}}{h_X^2 h_Y^2}\right]}$$

All correlations were estimated using linear regression models that initially included the main effects of temperature and population and an interaction between them, with interaction and population terms removed if they were found to be non-significant. In the majority of cases, population was not significant and this allowed for one correlation value per species.

2.4 Results

Mean trait values differed significantly between thermal environments for each species and generation ($P = < 0.001$). Rearing in a stressful thermal environment resulted in lower fecundity (Appendix A Table 1 and Appendix A Figure 2), smaller wing size (Appendix A Table 2 and Appendix A Figure 3), and a rounder, less elongated wing shape when adjusted for size (Appendix A Table 3 and Appendix A Figure 4) across all species, populations, and generations.

How does genetic variation change in a stressful thermal environment?

Fecundity

Phenotypic variation in fecundity did not differ significantly between thermal environments, but was slightly higher within the stressful environment than the benign environment (Table 2.1). CV_A estimates could not be calculated for *D. birchii* within the stressful temperature because offspring did not emerge in this treatment (the environment may have been too stressful). CV_A and evolvability (I_A) estimates for fecundity were higher than for morphological traits in all instances, and slightly higher in the stressful environment than in the benign environment in *D. serrata* (Table 2.1 and Fig. 2.2). Fecundity was found to have a low heritability overall (Table 2.1 and Fig. 2.2).

Morphological wing traits

Phenotypic variation in wing size was significantly higher within the benign environment than in the stressful environment for dams of both species (*D. serrata*: $P = 0.02$, *D. birchii*: $P = 0.005$; Appendix A Table 2). Heritability, evolvability, and CV_A estimates were higher within the benign environment than the stressful environment in *D. serrata*, and heritability values were overall much higher for wing size compared to fecundity (Table 2.1 and Fig. 2.2).

Phenotypic variation in wing shape variables significantly differed between thermal environments for all RW scores in *D. serrata* ($P < 0.005$ for all RW scores), but did not differ between thermal environments in *D. birchii*. The direction and magnitude of changes in phenotypic variances did not show a consistent pattern across thermal environments (Appendix A Table 3). Wing shape heritability increased within the stressful environment in *D. serrata* (Table 2.1 and Fig. 2.2), but the individual RW scores exhibited the opposite pattern (Appendix A Table 3). Heritability in all instances was much higher than for the fitness trait (fecundity). In addition, wing size and wing shape evolvabilities and CV_A estimates were all very low compared to the fitness trait (Fig. 2.2).

Table 2.1. Expression of genetic variance parameters for fecundity, wing size, and wing shape; including heritability (h^2), the coefficient of additive variance (CV_A), and evolvability (I_A). Phenotypic (V_P), additive (V_A) and residual (V_{res}) variances are also shown for the pooled dam and daughter values. Population was not a significant contributor to variance, so one metric was calculated per species from parent-offspring regressions. Bold values indicate a slope significantly different than zero and asterisks indicate significance level after correction for False Discovery Rate († $P < 0.1$; * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$). Parameters could not be calculated for *D. birchii* within the stressful environment because daughters did not develop. CV_A and I_A values shown are x 10^2. Values for individual relative warp scores for wing shape can be found in Appendix A Table 3.

		Benign (23ºC)							Stressful (28 ºC)						
Trait	Species	*N*	h^2 ± *SE*	V_P	V_A	V_{res}	CV_A	I_A	*N*	h^2 ± *SE*	V_P	V_A	V_{res}	CV_A	I_A
Fecundity	*D. birchii*	86	0.148 ± 0.116	1384.6	204.92	1179.68	15.88	2.524	-	-	-	-	-	-	-
	D. serrata	69	0.052 ± 0.124	1105.1	57.47	1047.64	5.26	0.276	65	0.040 ± 0.139	1879.5	75.18	1804.32	10.17	1.032
Wing size	*D. birchii*	81	**0.476** ± 0.131†	0.0005	0.0002	0.0003	0.224	0.0005	-	-	-	-	-	-	-
	D. serrata	64	**1.000** ± 0.142***	0.0005	0.0005	0.0000	0.324	0.0011	62	0.226 ± 0.124	0.0005	0.0001	0.0004	0.156	0.0002
Wing shape	*D. birchii*	81	**0.516** ± 0.22****	-	-	-	-	-	-	-	-	-	-	-	-
	D. serrata	64	**0.517** ± 0.25***	-	-	-	-	-	62	**0.599** ± 0.26*	-	-	-	-	-

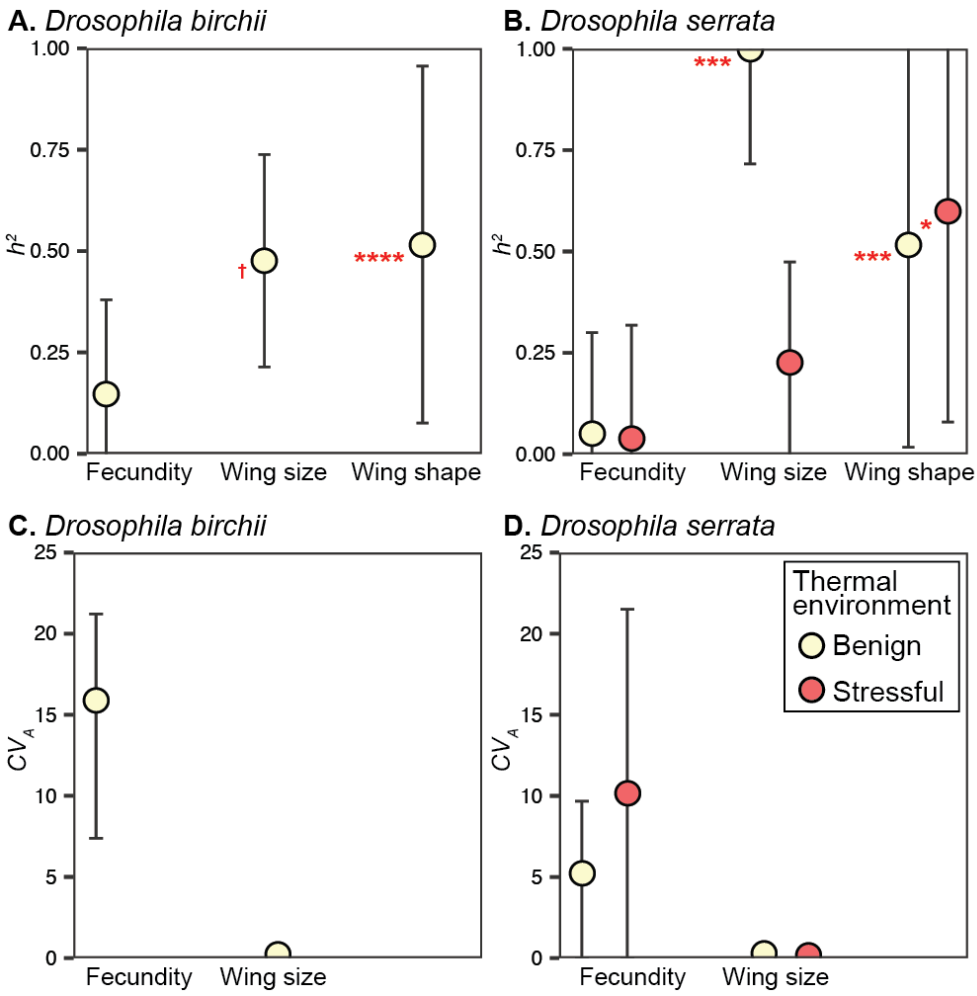

Figure 2.2: Heritability (h^2) and coefficient of additive variance (CV_A) values for a life history trait and morphological traits across a benign (23°C) and stressful (28°C) thermal environment. Two standardized estimates of additive genetic variance are shown for a life history and two morphological traits in two closely-related species of *Drosophila*. (**A**, **B**) Heritability is standardized by the total genetic variance and (**C**, **D**) coefficient of additive genetic variance is standardized by the trait mean. Evolvability (not shown) will exhibit the same pattern as CV_A. Standard errors (2x) are shown as error-bars, and asterisks indicate significance of the estimate after correction († $P < 0.1$; * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$). Standard errors for CV_A were calculated using the standard error estimates from heritability (see Appendix A Tables 1, 2 for details).

How does phenotypic correlation of traits change in a stressful environment?

There were no significant phenotypic correlations found between fecundity and wing size after correction for multiple comparisons by the False Discovery Rate method (Benjamini & Hochberg, 1995; Table 2.2 and Fig. 2.3). However, using a combined probabilities approach (i.e., a weighted *Z*-test), I did find a significant phenotypic correlation between wing size and

fecundity under benign conditions in *D. birchii*. Population was found to be a significant contributor to variation for the dams of *D. birchii*, so phenotypic correlations were also calculated and presented for each population separately (Table 2.2).

Table 2.2: **Phenotypic correlations between fecundity and wing size.** r_P is the phenotypic correlation and the *P*-values were obtained from an *F*-test of the linear regression of one trait on the other and both unadjusted (raw) and adjusted (corrected for using the False Discovery Rate method (Benjamini & Hochberg, 1995) are shown. Sample sizes (*N*) indicate the number of individuals used in each correlation. In addition, the combined probabilities from the dam and daughter generation are shown and were calculated using the weighted *Z*-test of combined probabilities.

		Benign (23ºC)				**Stressful (28 ºC)**			
Species	Generation Population	*N*	r_P	*P*-value (raw)	*P*-value	*N*	r_P	*P*-value (raw)	*P*-value
D. birchii	Dams	86	0.30	0.168	0.437	78	0.22	0.346	0.647
	Mt. Lewis	45	-0.14	0.655	0.763	-	-	-	-
	Paluma	41	0.76	0.014*	0.104	-	-	-	-
	Daughters	87	0.58	0.007**	0.073	-	-	-	-
	Combined probabilities		-	0.008**	-	-	-	-	-
D. serrata	Dams	78	-0.14	0.526	0.760	77	-0.18	0.422	0.707
	Daughters	67	0.12	0.628	0.763	65	0.52	0.035*	0.202
	Combined probabilities		-	0.603	-	-	-	0.345	-

Fecundity and wing shape traits exhibited mixed and inconsistent results (Fig. 2.3 and Appendix A Table 4). There was only one significant phenotypic correlation found between fecundity and a wing shape variable within the daughter generation of the Mt. Lewis population of *D. serrata* under a stressful thermal environment. Allometry was found within the benign environment for the daughter generation of *D. serrata*, indicating wing size and wing shape in this instance are still slightly correlated even after removing effects of size during the GPA analysis (Appendix A Table 4).

How do genetic covariances and correlations change with environmental stress?

There were no significant genetic covariances found between any of the traits (Appendix A Table 5). Additionally, there was no consistent trend detected for genetic covariances between species and thermal environments, and there was a general lack of pattern across populations, species, and thermal environments.

Further, there was no consistent pattern in genetic correlations (i.e., genetic covariances standardized by individual trait covariances) between species and thermal environments. Genetic correlations and their standard errors are shown in Figure 2.3 (and Appendix A Table 6). Genetic correlations in *D. birchii* were generally low ($-0.32 < r_G < 0.46$), while *D. serrata* traits exhibited high positive and negative genetic correlations, but this was not consistent across environments (Fig. 2.3).

I did find highly negative and highly positive genetic correlations between fecundity and wing morphometries in *D. serrata* (including values of ±1.00). However, these often had very wide standard errors and were not always significant. In the benign environment for *D. birchii*, I found a significant positive correlation between wing size and a wing shape variable (RWb-2; $r_G = 0.46 \pm 0.14$ SE, $P < 0.01$). In the benign environment for *D. serrata*, I found a significant negative genetic correlation between fecundity and wing size ($r_G = -1.00 \pm 0.08$ SE, $P < 0.001$) and a significant positive (RWs-1; $r_G = 0.75 \pm 0.16$ SE; $P < 0.0001$) and negative correlation between fecundity and a wing shape variable (RWs-4: $r_G = -0.92 \pm 0.10$ SE, $P = 0.0001$). In the stressful environment for *D. serrata*, I found a significant positive correlation between fecundity and wing size ($r_G = 0.84 \pm 0.29$ SE; $P < 0.05$) and fecundity and a wing shape variable (RWs-4; $r_G = 1.00 \pm 0.13$ SE; $P < 0.0001$; Fig. 2.3).

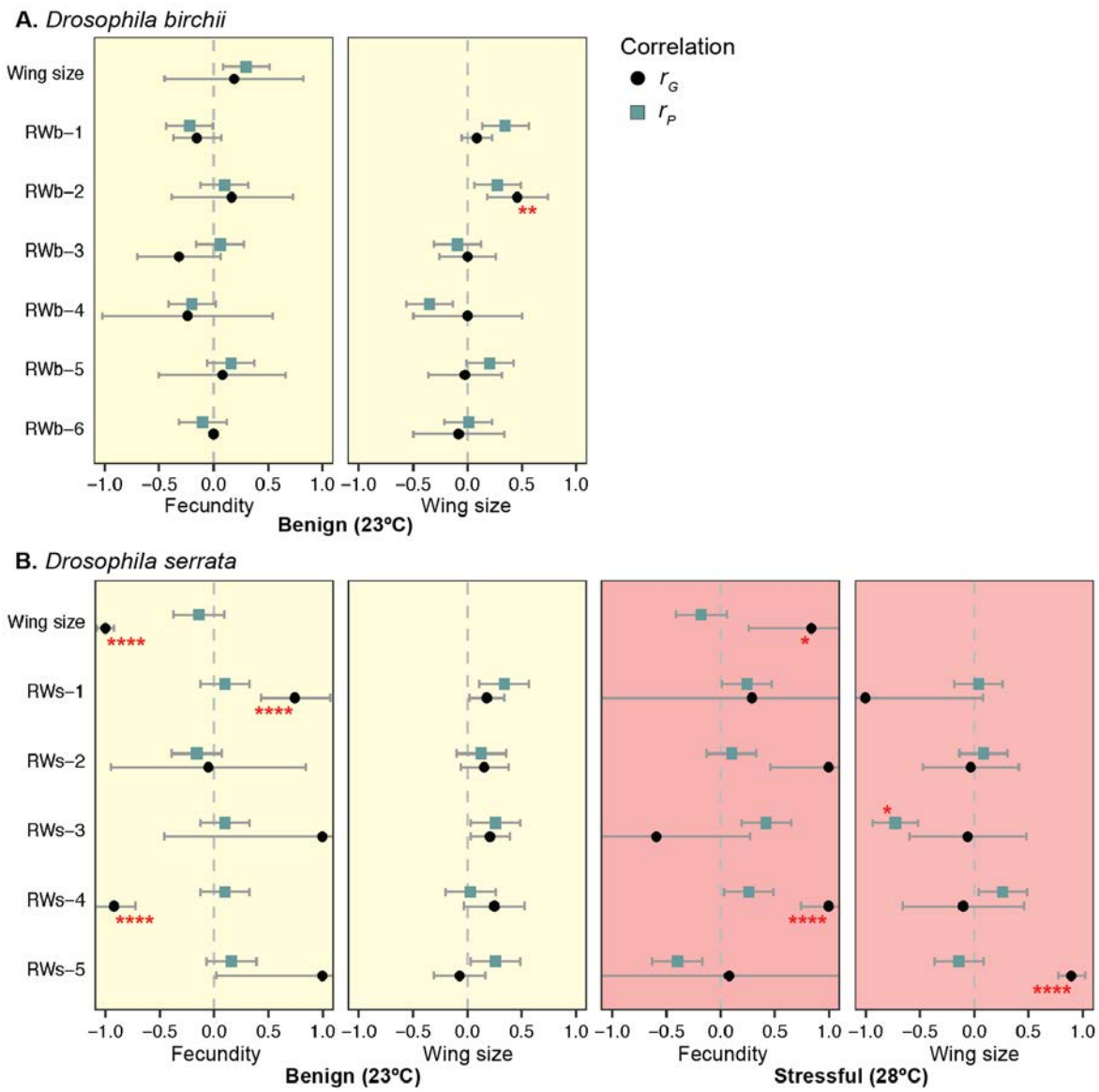

Figure 2.3. **Genetic and phenotypic correlations for fecundity and wing morphology in two sibling-species of *Drosophila*.**

Genetic correlations (r_G) and phenotypic correlations (r_P) between the trait on the x-axis (fecundity and wing size) and the trait on the y-axis (wing size and wing shape RW scores) across a benign and stressful thermal environment. Standard errors (2x) for the correlations are indicated by the grey error bars. Asterisks (in red) denote the correlation is significantly different from 0, obtained from the *z*-statistic calculated from standard errors (Altman & Bland, 2011) and *P*-values have been adjusted by the False Discovery Rate (* $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$). Phenotypic correlations shown are from the dam generation.

2.5 Discussion

The amount of genetic variation in a trait is important for predicting responses of populations and species to climate change as it determines the extent to which a trait can evolve via selection. However, genetic variance and heritability change between environments (Falconer & Mackay, 1996; Hoffmann & Schiffer, 1998; Sgrò & Hoffmann, 1998a; Sgrò & Hoffmann, 1998b; Hoffmann & Merilä, 1999) potentially due to increased environmental variance and reduced additive genetic variance, genotype-by-environment interactions that affect cross-environment genetic correlations, and cryptic genetic variation (Fischer et al., 2020). It is essential to recognise and incorporate this into climate change adaptation research (Shaw, 2019), but consistent and predictable patterns have not been detected. It is unclear whether such patterns exist or whether genetic variance and heritability must always be considered in the context of specific traits, populations and environments. Here, I showed that temperature stress can alter the heritability, coefficient of additive genetic variation, and evolvability, of both fecundity and morphological traits in two closely-related species of *Drosophila* (one being a generalist and one being a specialist). However, I found no consistent pattern in the direction of change in additive genetic variance and phenotypic and genetic covariances across thermal environments.

First, I confirmed that the warmer (‘stressful’) thermal environment did indeed induce stress in both species, as demonstrated by lower fecundity, smaller size, and a significantly different wing shape (Appendix A Tables 1–3 and Appendix A Figures 2–4). In addition, the specialist species (*D. birchii*) failed to develop offspring within the stressful thermal environment. Although there were no experimental differences between the dam and daughter generation that might have caused this, it is possible that maternal effects induced by development within a stressful environment prevented the production of viable offspring. This could alternatively be a paternal effect as it has been shown that *D. birchii* sperm is very sensitive to thermal stress during development (Saxon et al., 2018b), and because I attempted to control for maternal effects by developing the initial parental generation (i.e., P in Fig. 2.1) in the same thermal environment as dams and daughters. Although not a direct aim of this paper, measuring the viability of offspring within a stressful environment is relevant to many evolutionary studies (both in the laboratory and in the field). This is because many studies estimate fitness by measuring the number of offspring directly, but the viability of those offspring is what will maintain the long-term fitness of a population.

Second, I found lower heritability in the stressful compared to the benign thermal environment for fecundity and wing size, although not in wing shape in *D. serrata* (Table 2.1 and Fig. 2.2B). This corroborates a large number of previous studies that show heritability declined under stressful conditions (e.g., Hoffmann & Schiffer, 1998; Kristensen et al., 2015; and reviewed in Hoffmann & Parsons, 1991; Hoffmann & Merilä, 1999; Charmantier & Garant, 2005; Rowiński & Rogell, 2017). This has important implications for species living close to their upper thermal limits (like many species in the tropics; Deutsch et al., 2008; Kingsolver et al., 2013) because even a small change in environmental conditions may induce a large amount of stress, and these results suggest adaptive potential is reduced under stressful temperatures.

However, heritability has been shown to have inherent issues when comparing between environments, as non-additive genetic and environmental variation contribute to it (Houle, 1992). To address this problem, I also investigated CV_A and I_A. These are often more appropriate estimates to use when comparing genetic variation and evolvability across traits and environments, as they are not affected by non-additive sources of environmental variance (Houle, 1992; Bubliy & Loeschcke, 2002; Garcia-Gonzalez et al., 2012; but see Hoffmann et al., 2016 for stipulations). Specifically, while heritability tells us the absolute difference in a trait mean from one generation under selection to the next, I_A represents a percentage change in a trait that is expected under a given strength of selection (Hansen et al., 2003; Hansen et al., 2011; Garcia-Gonzalez et al., 2012). CV_A and I_A were higher under the stressful environment for fecundity, while the opposite was true for wing size (Fig. 2.2D). Therefore, while the heritability values suggest that the response to selection on fecundity and wing size will decrease under stressful temperatures, CV_A and I_A suggest that fecundity has greater relative evolutionary potential under the stressful environment than the benign environment and the opposite is true for wing size (Fig. 2.2). Although it seems heritability and CV_A values may be contradictory, it could be that while the absolute change in fitness (i.e., heritability) will be less in the stressful environment for fecundity, there will be a greater relative increase in fitness in the stressful environment because mean fitness is lower—but this could result in a smaller absolute change in trait mean thus corroborating the heritability results. However, CV_A and I_A values are important metrics to consider because they will always change in the same direction as V_A. Alternatively, heritability does not necessarily change in the same direction as V_A because factors that increase V_A often increase total variance, which in turn will decrease heritability.

An increase in additive genetic variance under stressful temperatures for the measured fitness trait (i.e., fecundity) is advantageous for these *Drosophila* species, both of which live near critical thermal limits (Kellermann et al., 2009; Overgaard et al., 2011). Interestingly, these results are consistent with a recent meta-analysis (Rowiński & Rogell, 2017), which showed that the coefficient of genetic variance (CV_A) was higher under stressful conditions for life history traits but not for morphological traits. In terms of wing size, it should be noted that the measured CV_A differed from values previously measured in *D. birchii* (Kellermann et al., 2006); where CV_A was relatively two-fold higher than what I found here. However, in the previous experiment (Kellermann et al., 2006), wing size was measured from flies reared in a benign environment at 25°C (compared to the benign environment measured in this study at 23°C); potentially indicating that even a slight difference in thermal environments can affect estimates of genetic variance. The reported V_A and V_P values indicate differences in CV_A between this study and Kellermann et al. (2006) are due to an increased V_A in their study and not a difference in trait mean that could also induce larger CV_A values (if the trait mean was lower). Collectively, this, along with the other results discussed here, reveal that environmental interactions (that are included in estimating heritability but not CV_A and I_A), potentially play a very large role in shaping the amount of additive genetic variance that selection can act upon.

Overall, the heritability values are similar to those reported for fecundity, wing size, and wing shape for *Drosophila* in the literature (e.g., Gilchrist & Partridge, 1999; Hoffmann & Shirriffs, 2002; Moraes et al., 2004; Kellermann et al., 2006). Additionally, I examined the differences in genetic variation between fecundity and morphological traits, since patterns in heritability and additive variance (CV_A and I_A) were contradictory. I found that heritabilities were higher for the wing morphology traits than for fecundity (Fig. 2.2A, B). This coincides with the majority of literature that show morphological traits often have higher heritabilities than life history traits (Mousseau & Roff, 1987; but for opposing example see Sgrò & Hoffmann, 1998a). In direct contrast to this, CV_A and I_A were both magnitudes larger for fecundity than what was found for wing morphology (Fig. 2.2C, D). This finding supports theory proposed by Houle (1992); that life history traits may have a higher evolvability than morphological traits. This may indicate that fecundity can show a greater response to selection in this case. Under the benign environment, CV_A and I_A for fecundity were more than 94% higher than for wing size in both species, and in the stressful environment, fecundity exhibited a CV_A and I_A that was approximately 80% higher than for wing size for *D. serrata* (Fig. 2.2).

The low heritability values detected for fecundity are consistent with classic theory that suggests ultimate fitness traits will exhibit low heritabilities due to directional selection that fixes beneficial alleles and erodes additive and residual variance (Mousseau & Roff, 1987; Falconer & Mackay, 1996; Merilä & Sheldon, 1999). However, in direct contrast to this, I found that additive variance was actually significantly higher in fecundity where h^2 was low. When examining residual variance ($V_{res} = V_P - V_A$), it becomes evident that increased residual variance is responsible for a reduced heritability in fecundity, rather than eroded additive genetic variance (Kruuk et al., 2000; Merilä & Sheldon, 2000; McCleery et al., 2004; Moraes et al., 2004; Table 2.1). In a study examining how residual and additive variance contributes to heritability values across fitness and morphological traits, Merilä & Sheldon (1999) found fitness traits generally exhibit a higher residual variance compared to morphological traits due to an accumulation of non-additive genetic and early environmental effects. These results support their findings and emphasize the importance of considering trait type when examining how selection shapes additive genetic variance.

An additional aim of my study was to determine whether an easy-to-measure morphological trait can be used as a proxy for fecundity across environments. To examine this, I looked at phenotypic and genetic correlations between fecundity and wing morphology. Although it has been shown that wing length correlates with fecundity in benign environments (Chiang & Hodson, 1950; Tantawy & Vetukhiv, 1960; Santos et al., 1992; Woods et al., 2002), recent studies have found both evidence for (Woods et al., 2002) or a lack of evidence for (Sgrò & Hoffmann, 1998b) positive relationships between wing length, wing width, and fecundity in stressful environments. Here, unadjusted significance tests are suggestive of significant phenotypic correlations between fecundity and wing size in the benign environment for one population of *D. birchii* dams and for *D. birchii* daughters; and in the stressful thermal environment for *D. serrata* daughters. However, these became insignificant after I corrected for False Detection Rate (see Table 2.2), False Detection Rate is a conservative method for multiple comparison in terms of type II errors. As such, I also combined the probabilities to increase power of the significance test. The combined probabilities method indicates that wing size and fecundity are significantly phenotypically correlated in the benign environment for *D. birchii*, meaning we can use wing size as a proxy for fecundity for this population in benign (but not stressful) conditions.

Phenotypic correlations between fecundity and wing shape differed in both sign and magnitude across environments (Appendix A Table 4). There was no trend to where significant phenotypic correlations occurred and relationships between fecundity and wing

morphology could not be generalised across thermal environments, species, or even between generations.

Genetic correlations were all fairly low in *D. birchii*, but highly-positive and highly-negative correlations were found in both environments for *D. serrata* (Fig. 2.3 and Appendix A Table 6). Most interestingly in *D. serrata*, fecundity and wing size were significantly negatively-correlated in the benign environment and significantly positively-correlated in the stressful environment. A significant genetic correlation between a pair of traits suggests that the traits are genetically associated through linkage or pleiotropy (influenced by a common locus or loci; Wilson et al., 2010). However, a change in the magnitude or sign of genetic correlations across environments suggests that this genetic association is environment-specific (Falconer & Mackay, 1996; see Gutteling et al., 2007 for example). So, while a positive correlation between fecundity and wing size in the stressful environment may indicate that the same gene underlies both traits or the genes influencing both traits are in linkage disequilibrium (Wood & Brodie, 2016); a negative correlation in the benign environment may indicate antagonistic pleiotropy between them if this data was looked at independently. However, the drastic change between thermal environments suggests there are environment-specific gene effects that affect these correlations.

In addition, the sign and magnitude of genetic correlation values between fecundity and wing shape variables differed between thermal environments in an inconsistent way. Also, when examining phenotypic correlations and genetic correlations together, I did not find phenotypic correlations that were similar to significant genetic correlations (Fig. 2.3). This suggests that the environment is masking phenotypic correlations. The large standard errors associated with many of the genetic correlations also suggest that I may lack sufficient power to detect genetic correlations in some cases. Very large sample sizes are needed in quantitative genetic experiments to estimate heritabilities and genetic correlations with a high degree of precision (Roff, 1995; Falconer & Mackay, 1996). This is hard to achieve due to logistical challenges, and may potentially explain why there is so much variation across species, populations, and traits in effects of environment on heritability and genetic correlations in the literature (reviewed in Garcia-Gonzales et al., 2012; Hansen et al. 2011).

Future directions

Here, I found that genetic variance and phenotypic and genetic correlations change across thermal environments. However, the direction of these changes was not always consistent

across traits, closely-related species, populations within a species, or even generations. This suggests that researchers need to examine adaptive potential specific to their environment, species, and populations if they hope to obtain accurate parameters to predict evolutionary potential. The type of data collected here should represent a starting point for researchers aiming to do so.

Additionally, researchers need to be aware that high genetic variation does not necessarily indicate an increased evolutionary response. Although it is assumed that selection has a strong effect when genetic variation is high and a weak effect when genetic variation is low (when all other factors remain the same), there has been limited evidence showing how they interact and the studies that have looked at their relationship report a fairly weak association (Wood & Brodie, 2016; Ramakers et al., 2018). Future research needs to consider how evolutionary potential is affected by the environment. I show here that genetic variance is highly dependent on temperature and it is accepted that selection is directly mediated by the environment. Yet, specifically in terms of stressful temperatures, a meta-analysis on how selection and genetic variance are coupled found temperature is likely to affect the amount of genetic variation in a population more than the strength of selection (Wood & Brodie, 2016). Wood and Brodie (2016) found that temperature affected the amount of genetic variation and the strength of selection in both morphological and fitness traits asymmetrically; meaning the measured impact of temperature stress on genetic variation does not necessarily predict the magnitude of the evolutionary change. Researchers should examine how both genetic variance and selective force (both strength and directionality of selection) is influenced by specific environments to determine the adaptive potential of species to climate change. If a highly positive correlation exists between the two, environmental change would increase both, directly causing increased adaptation; and predictions on how species will adapt to changing environments would be more straightforward (Wood & Brodie, 2016; Ramakers et al., 2018; Fischer et al., 2020).

However, genetic correlations also need to be considered in this context. A negative genetic correlation between two traits will constrain evolution on one trait even with an increase in genetic variation and a positive selection differential (and vice versa; Conner, 2012; Wood & Brodie, 2016). An additional consideration is that the underlying genetic architecture of the trait (polygenic or large-effect loci) should be considered. For example, polygenic traits have been shown to produce greater long-term population viability than in traits affected by large-effect alleles when heritability and the selective force is constant (e.g., Kardos & Luikart, 2021). Generally, life-history traits are thought to be polygenic in

comparison to large-effect phenotypic traits related to morphology, indicating another reason why trait type needs to be considered when investigating adaptive potential.

In conclusion, although I present clear evidence that stressful temperatures affect genetic variation, I did not detect a consistent pattern to that change. These results suggest that adaptive potential cannot be generalised across environments, closely-related species or populations and needs to be considered on a case-by-case basis, specific to the trait in question, and by using a multivariate approach.

2.6 Highlights

- I assessed how a stressful thermal environment affects genetic variance in two species of *Drosophila.*
- I found that genetic variances, heritability, and phenotypic and genetic correlations change across thermal environments, but in no consistent way.
- Researchers need to use genetic variance values specific to the trait in questions and specific to their species and population to accurately predict adaptation potential.

Chapter 3: Adaptable temperature array for characterising ecological and evolutionary effects on thermal physiology.

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Contributions: JC, MH, and CH conceived the equipment. Design and development was done by the JCU Innovation centre as follows: WM designed the equipment, with design specifications from JC and MH; and RW wrote the operational coding for the equipment. JC and LE built and validated equipment. JC performed tests and analysed data. JC, MH, and CH wrote the manuscript. All authors gave final approval for publication.

3.1 Abstract

1. To accurately characterise a species' thermal niche and aid in predicting effects of climate change we must not only include information on thermal tolerances and physiological responses to changing temperatures, but also incorporate ecological effects and evolutionary processes that may shape a species' niche. However, quickly and practically collecting data on key factors such as adaptation potential, behaviour, effects of species interactions, plasticity, and thermal tolerances is logistically challenging.
2. I have therefore created an adjustable temperature array (ATA) to assist with experimental ecology and evolution research. ATA's are a row of independent temperature points controlled and set by the user and made from commercially-available parts. This allows the user to create unique thermal landscapes relevant to their study organism(s) and question(s). Further, the option of using an enclosed cage allows the user to answer questions at the individual, population, or community level in the context of changing thermal environments. ATA's are able to be user-set to constant or dynamic temperature regimes and are designed for use on small animals (e.g., fruit flies, beetles, mosquitoes) or plants (e.g., germinating seeds).
3. I have tested and confirmed the accuracy of the ATA to several thermal landscapes that would be useful for experimental ecology and evolution, including: 1) coarse resolution of a broad thermal niche ranging from 12° to 42°C in 2°C intervals (R^2 = 0.998); 2) fine resolution of a narrow thermal niche ranging from 15° to 32°C in 1°C intervals (R^2= 0.997); 3) a pyramid-shaped niche consisting of a gradient from 14° to 30°C in 2°C intervals (R^2 = 0.997); and 4) a very narrow thermal niche with replicate thermal resources ranging from 26.5° to 34°C in 1.5°C intervals (R^2 = 0.989).
4. The equipment described here is an important tool for thermal niche studies and will aid in gathering information on effects of ecological and evolutionary processes to create a comprehensive picture of species responses to climate change.

3.2 Introduction

Temperature is widely accepted as a critical abiotic factor affecting species abundances and distributions (Cossins & Bowler, 1987; Angilletta, 2009; Overgaard et al., 2014). With global temperatures predicted to rise in the next century (IPCC, 2019), accurately understanding the limiting factors for a species' current distribution, as well as predicting how species will respond to changing temperatures is crucial. Hence, we must comprehensively understand a species' thermal niche. First, a species' thermal niche should include its full physiological tolerances and thermal limits. Second, predictions should account for ecological effects and evolutionary processes that are seldom incorporated into predictions (Williams et al., 2008; Hoffmann & Sgrò, 2011; Urban et al., 2012; Bush et al., 2016). This includes incorporating effects of biotic interactions, behavioural responses and preferences, and as well as the potential for acclimation, adaptation, and plasticity. Currently, solutions on how to accurately capture the full thermal niche and quickly and practically collect data on eco-evolutionary processes have proven difficult (Pearman et al., 2008; Thuiller et al., 2013). Field observations may not capture the full niche space and experimentally testing these factors requires a large number of replicates over climate space, and extended time periods to test for adaptation.

I have therefore created an adjustable temperature array (ATA) to experimentally capture a species' thermal niche. ATAs are a row of individual temperature points set by the user. Temperatures can range from 12°–42°C as tested here (but theoretically can range from −55°–85°C). The ATAs herein were designed to be used on small ectotherms in a controlled laboratory setting, but can be used on a wide variety of ectotherms or small endotherms with appropriate re-sizing of the temperature points, or for growing small plants, fungi or algae, or germinating seeds. Our ATAs can be used with two different experimental setups: closed vials (e.g., for testing physiological performance and limits, and acclimation and plasticity responses) or open cage (e.g., for examining thermal behaviour and preferences, and biotic interactions); and can be programmed with a constant or dynamic temperature regime (i.e., ramping or fluctuating temperatures). The versatility of the experimental setups allows users to create a comprehensive picture of a single species' thermal niche with the advantage of completely individualized, replicated, and randomized environments to reduce confounding effects of pseudoreplication and other problems inherent to using temperature cabinets.

3.3 Materials and Methods

Each ATA consists of a temperature strip, control box, and cage, all of which can be disconnected for set up and maintenance. All equipment and parts were purchased from consumer hardware, electronic, and online retailers (Appendix B Supplementary Material 1).

Temperature Strip

The temperature strip is comprised of two parts: 1) the temperature points where each is controlled to a user-specified temperature and, 2) a water-cooling system to remove excess heat from the system.

Temperature points

The ATAs designed herein contain 18 temperature points, although users can create ATAs to contain a quantity more relevant to their study question. The temperature points create individual temperature 'spots' that are user-specified. For example, a user investigating how a species performs along a thermal gradient would set each temperature point to a unique temperature along that gradient, a user examining threshold temperatures might set all temperature points to the same temperature to concurrently run replicates, and a user investigating eco-evolutionary dynamics might use real-world temperature readings to set each temperature point to a fluctuating temperature regime (i.e., with temperatures dipping at night and spiking during the day).

The components used to create 18 temperature points are listed in Table 3.1 and shown in Fig. 3.1. Aluminium heatsink bars were mounted end to end onto a stable baseboard and each temperature point was made by attaching a heat pump and an aluminium vial holder (Figs. 3.1a, b). A single temperature sensor was then super-glued to each vial holder (Fig. 3.1c). For details of construction, see Appendix B Supplementary Material 2.

Table 3.1: Components for temperature points.

Component	Quantity	Specifications	Use
Baseboard	1	Size determined by number of temperature points	Mounts electronics and hardware
Aluminium water-cooled heat-exchange bar	3	1 per 6 temperature points with size determined by size of set-points	Provides a heatsink for heat pumps
7 W thermoelectric heat pumps (Peltier coolers)	18	1 per temperature point with a working temperature range of −55°–88°C (dimensions: 20 mm)	Heats and cools at temperature point
Aluminium machined vial holders	18	1 per temperature point designed to hold a standard 100 mm x 25 mm glass specimen vial; machined from solid aluminium bars	Conducts heat to/from heat pump and provides insulation to vials
Temperature sensors	18	1 per temperature point with −10°–85°C with ± 0.01°C resolution and ± 0.5°C uncalibrated accuracy (Dallas DS1820. Maxi3m Integrated. San Jose, CA)	Provides over/under temperature feedback to microcontroller
16-pin female plug-socket cables	3	1 cable provides connection for 6 temperature points	Provides connection to control box

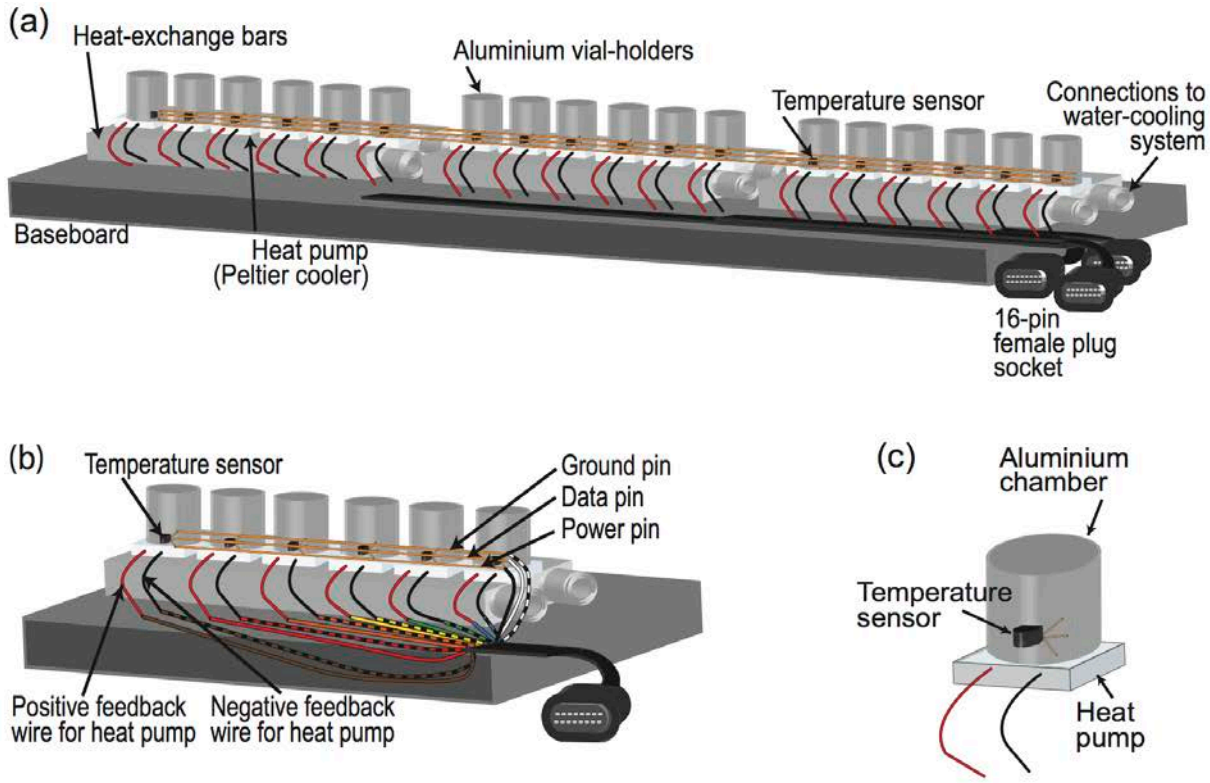

Figure 3.1: The design and placement of the temperature strip components can be seen in (**a**). A detailed schematic on the construction and wiring of one third of a temperature strip is shown in (**b**), and a detailed image of a single temperature point is shown in (**c**).

Water-cooling system

The water-cooling system uses a cold-water reservoir that pumps water through the aluminium heatsink bars to redistribute and strip excess heat away from the temperature strips and is necessary for the heat pumps' performance. The water-cooling system can be attached to a constantly flowing cold water tap (i.e., mains water) or a simple water pump if the user can keep reservoir water cold. For more information on the setup of the water-cooling system, see Appendix B Supplementary Material 2.

Control Box

The control box system is comprised of: 1) a closed container to hold electronics, 2) a power supply, 3) the internal electronics for each temperature point, and 4) a LED lighting indicator system.

Closed Container or Box

All components of the control box should be housed together in a semi-closed container to protect electronics and wiring. For example, each control box herein was set up inside an empty computer tower case as this already contained a power source for easy power supply.

Power Supply

Each ATA operates off mains (i.e., grid) power and draws a maximum power load of 72 W.

Control Box Internal Electronics

The control box internal electronics are shown in Table 3.2 and Fig. 3.2, with more information on circuit schematics in Appendix B Supplementary Material 3.

Table 3.2: Components for control box.

Component	Quantity	Specifications	Use
Embedded microcontroller	1	WiFi embedded development board	Controls ATA
Switching solid-state relays	9	1 per 2 temperature points	Turns heat pump on or off
16-pin male plug-socket cables	3	1 cable provides connection for 6 temperature points	Provides connection to control box

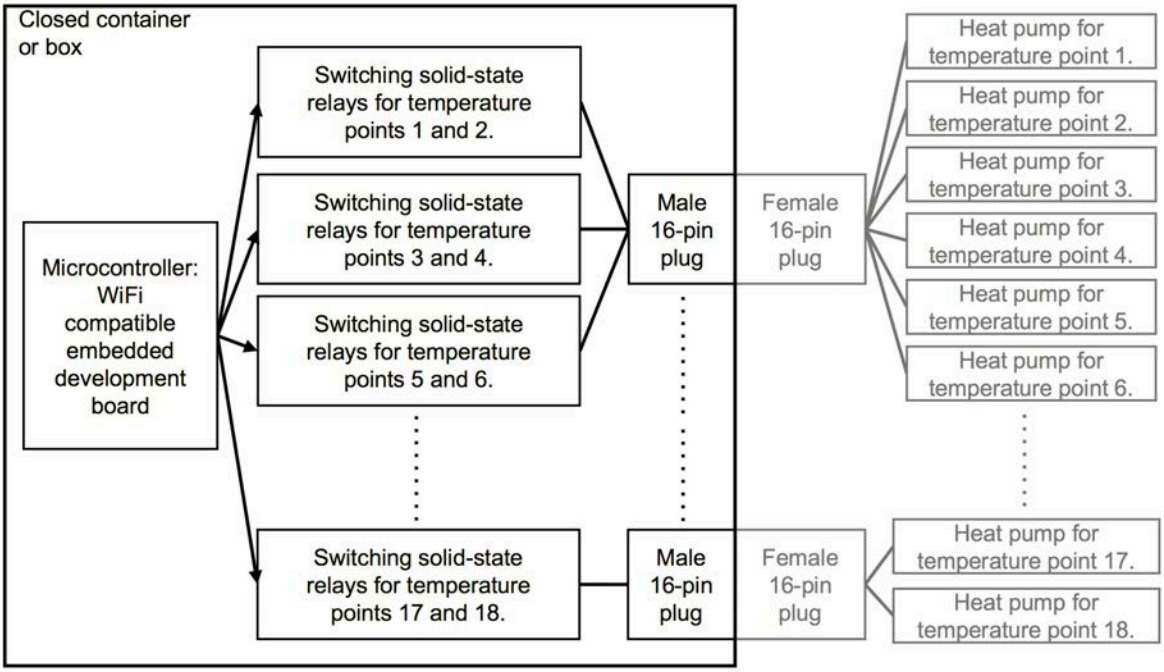

Figure 3.2: The control box internal electronics are shown above, along with how they connect to each other and relevant temperature strip components (shown in grey). The control box is connected to the temperature strip via 16-pin male plugs that plug into a corresponding 16-pin female plug on the temperature strip. For more details on control box components, setup, and wiring, see Appendix B Supplementary Material 3.

LED lighting indicator system

The exterior of the control box has eighteen tricoloured LED lights that correspond to each temperature point to provide a quick indication of temperature accuracy (Fig. 3.3 and Appendix B Supplementary Material 4).

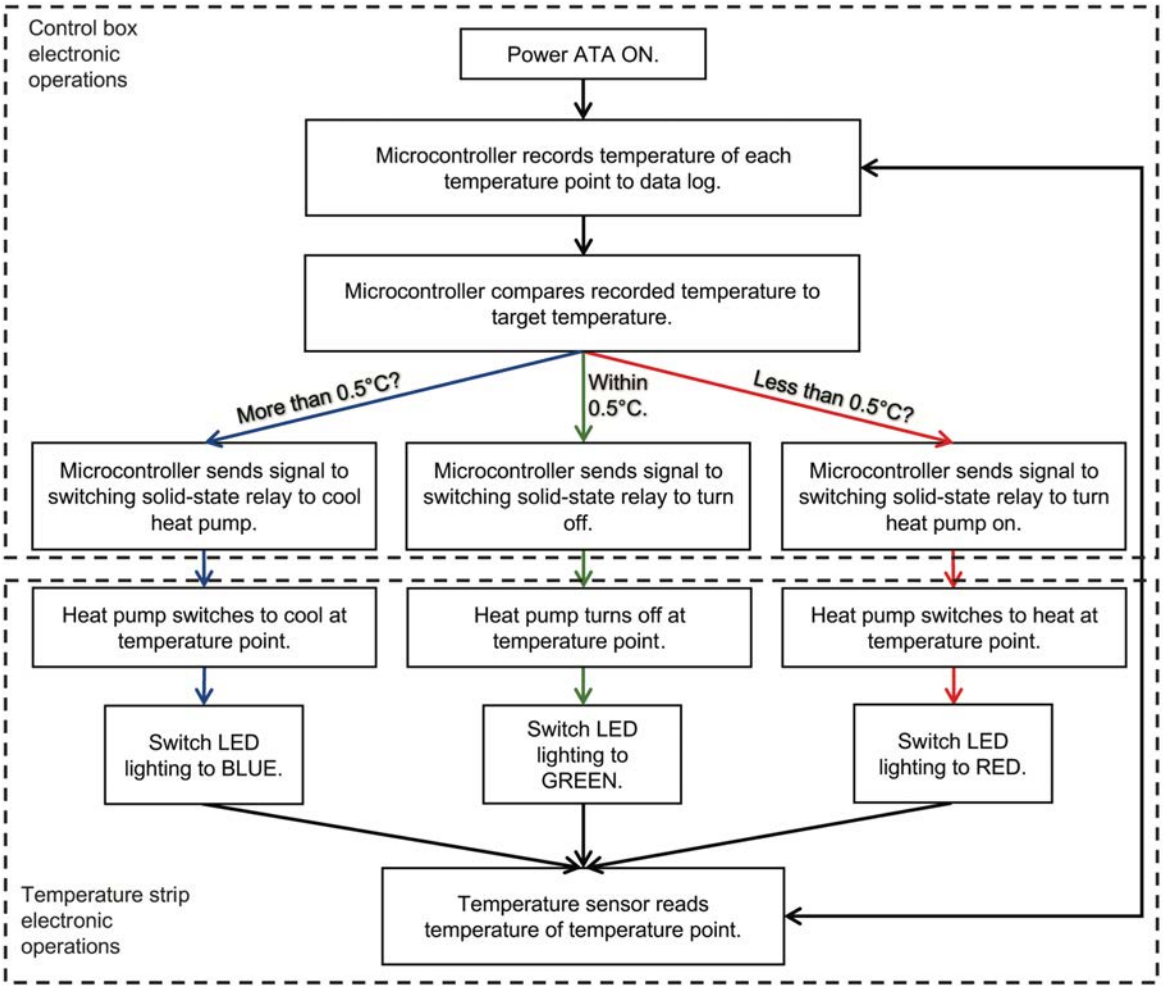

Figure 3.3: The operational process of an ATA.

Operational process

The operational process an ATA goes through after being turned on is shown in Fig. 3.3. Microcontrollers are programmed before being turned on with user-specified temperature regimes for each temperature point. In addition, the time period at which the microcontroller and temperature sensors read and record temperatures can be specified (e.g., every 30 seconds, 1 minute, etc.). The operational code needed to operate ATAs can be accessed from GitHub and uploaded using the open-source electronics platform Arduino (www.arduino.cc; Wheat, 2011); see data accessibility section).

Study organism experimental setup

Experiments can be conducted in two ways depending on the user's objective: closed vial or open cage. The closed vial design restricts organisms to specific temperature points by housing them in closed glass specimen vials (Fig. 3.4a). The open cage setup has individuals housed within a cage with open vials at temperature points for individuals to choose amongst (Fig. 3.4b). ATAs were built to fit standard 100 mm x 25 mm glass vials. Cages were built by using 34 L clear storage containers and drilling 25 mm holes into the containers to coincide with the location of temperature-point vial holders (Fig. 3.4b). Two additional large holes were cut into the side of the container and pantyhose were hot-glued to the sides to provide hand openings. These allow users to access the cage while providing a tight seal around the arm.

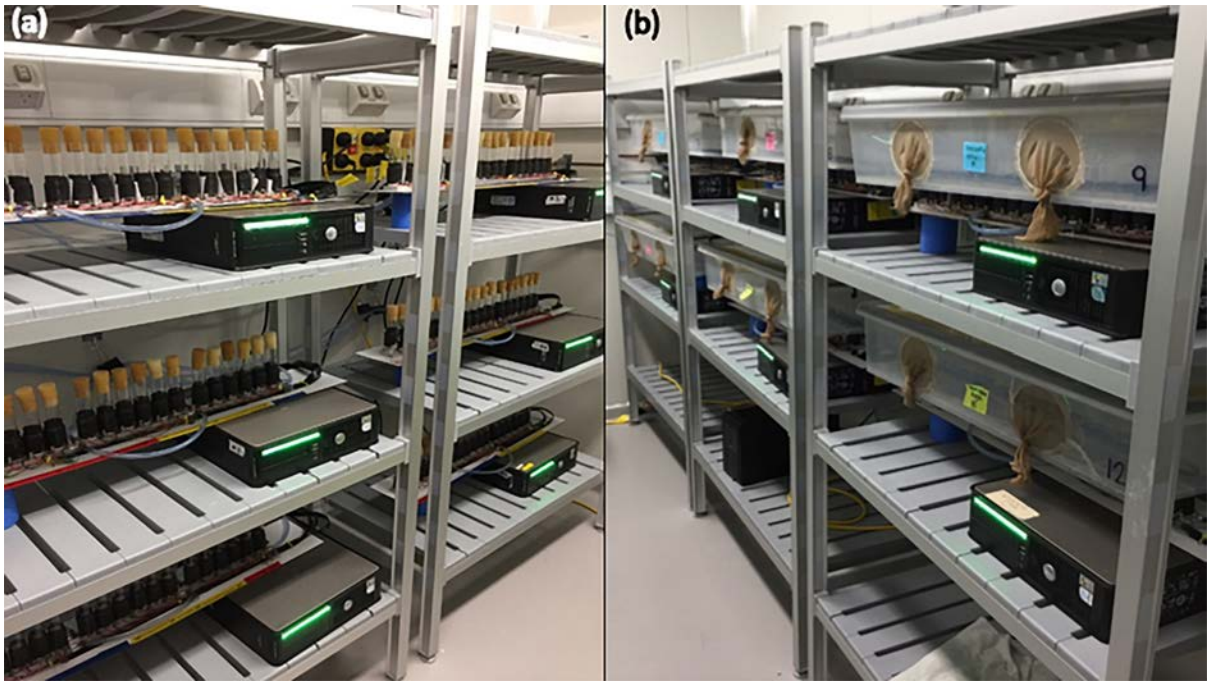

Figure 3.4: Images of (a) ATAs in a controlled temperature and humidity room with stoppered glass specimen vials in a closed vial setup, and (b) ATAs arranged with an open cage setup to run choice experiments. The LED lights on the front of each control box are green, indicating all temperature points are within 0.5°C of target temperature.

Validation tests

I arranged 24 ATAs in a controlled temperature and humidity room (23°C and 60% RH) and tested the accuracy of the ATAs to four constant thermal landscapes that may be useful in experimental ecology and evolution. Four ATAs were haphazardly chosen and tested per landscape. Details of each test are included in Table 3.3.

Table 3.3: Test details for four validation tests performed to confirm accuracy of ATAs.

Test: Niche landscape	Temperature gradient (°C)	Coarseness (°C)	Logging frequency	Duration	Example scenario:
A: Broad thermal niche	12°–42°C	Coarse: 2°C intervals	Every minute	7 days	To capture outer bounds of a fundamental thermal niche
B: Narrow thermal niche	15°–32°C	Fine: 1°C intervals	Every minute	7 days	To finely characterise a thermal niche from a mountain-top to the lowlands
C: Pyramid-shaped thermal niche	14°–30°–14°C	Coarse: 2°C intervals	Every minute	7 days	To investigate a transect from one mountain-top, through a valley, and up to a neighbouring mountain-top
D. Very narrow thermal niche	26.5°–34°C with each temperature replicated three times	Medium (with replication): 1.5°C intervals	Every 2 minutes	60 days	To examine a niche where different food resources may be available at each temperature

In addition, I tested how temperatures vary within a vial for three temperatures (i.e., 15°C, 25°C, and 36°C) under each experimental setup and with or without food resources. More information on these tests can be found in Appendix B Supplementary Material 5.

3.4 Results

Correlation, linear regression, and root-mean-square-error analyses were performed in R (R Core Team, 2019) for each test landscape to evaluate the reliability and accuracy of ATAs. In addition, accuracy measures are shown by the mean deviation, standard deviation, and the single furthest deviation of any temperature point in the test landscape (Table 3.4 and Appendix B Supplementary Material 5).

Table 3.4: Results of accuracy and reliability analysis for validation tests. * indicates a significance level of $P < 0.0001$.

Test	Mean deviation (°C ± sd)	Single furthest deviation	Correlation test (*r*)	RMSE (°C)	Regression Fitted regression line	CI of slope	R^2
A	0.082 ± 0.302	2.8°C for 1 min at 12°C	0.996*	0.322	y = 0.973x + 0.808	[0.973, 0.973]	0.998
B	0.139 ± 0.276	2.0°C for 1 min at 15°C	0.998*	0.278	y = 0.983x + 0.519	[0.983, 0.983]	0.997
C	0.137 ± 0.255	1.7°C for 1 min at 14°C	0.994*	0.263	y = 0.983x + 0.624	[0.982, 0.983]	0.997
D	0.039 ± 0.265	2.8°C for 8 min at 31°C	0.986*	0.267	y = 0.984x + 0.543	[0.983, 0.984]	0.989

All ATAs (except one replicate from the pyramid-shaped thermal landscape, which failed due to a malfunction of the water-cooling system) proved to reliably maintain the target temperatures throughout the testing period. All tests showed a highly significant positive correlation to target temperature with a near 1:1 fit between the actual temperatures and the target temperatures. Results are shown in Table 3.4 and Fig. 3.5. Additional figures can be found in Appendix B Supplementary Material 5.

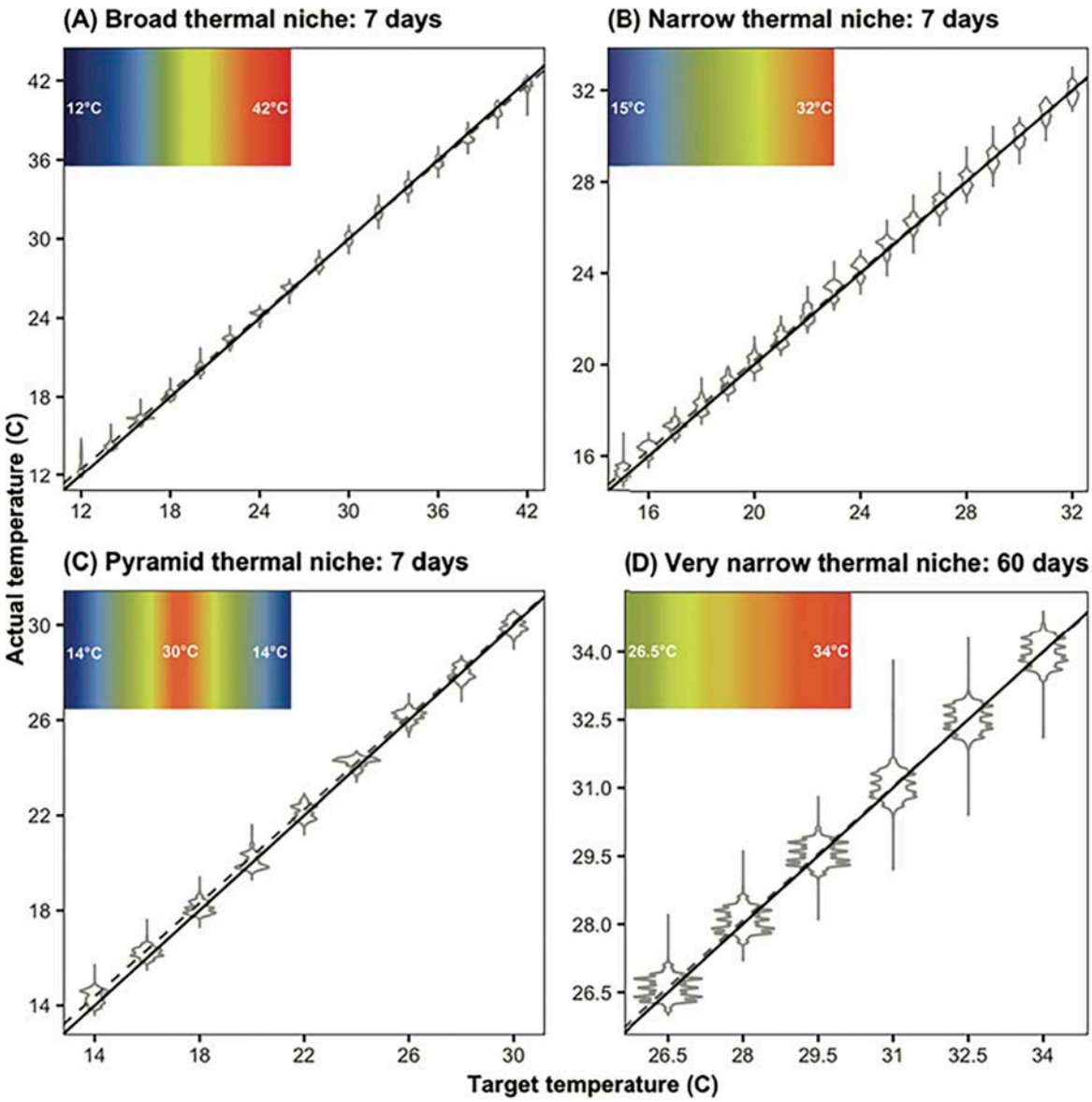

Figure 3.5: Results of the validation tests for several niche landscapes over short- and long-term durations. For each test, regression plots show a near 1:1 fit comparing the actual temperatures (grey violin plots) to the target temperatures. The dashed grey lines represent the regression line and the black lines are a line with slope of 1 for comparison of actual temperatures to target temperatures.

Tests examining how temperatures differ within the vial environment showed that temperatures at the bottom of the vial reliably maintained set temperatures (R^2=0.99, RMSE=0.79), but a thermal gradient away from the heating/cooling point was present within the vial depending on set temperature versus the room temperature. More information on the results of these tests can be found in Appendix B Supplementary Material 5.

3.5 Discussion

The validation tests demonstrated the ATAs described here can consistently maintain a variety of user-set thermal landscapes over an extended period of time. The temperature points functioned most accurately between 18°–34°C, with higher set-points resulting in actual temperatures lower than the set temperature and lower set-points resulting in temperatures higher than the set temperature (Fig. 3.5). This is not a limitation of the heating and cooling capacity of the equipment itself, which can theoretically go from −55°–85°C, but is an artefact of the user-specified 'throttling speed' of the heat pumps and therefore can be rectified in the operational code.

One ATA malfunctioned for a period of 7 hours during the test; however, the problem was with the water-cooling system. This problem could be fixed by setting up an alarm notification system for each ATA, which could be done using the WiFi compatible microcontroller to monitor the actual temperatures of each point remotely.

In addition, tests examining how temperatures differed within a vial indicated that a temperature gradient can occur as air that is heated or cooled from the bottom of the vial mixes with ambient air. This can be reduced by using insulation or by reducing the amount of vial space that is exposed to the ambient air (i.e., making aluminium chambers larger). Although I recognize this as a limitation, these tests showed temperatures on the bottom of the vial maintained set temperatures. This may not be an issue if users are studying ectotherms and using vials as thermal resource points, as most ectotherms will lay their eggs directly into the resource. Larvae and adults will also be exposed to bottom temperatures when ovipositing or feeding. If vials are used in open cage experiments, individuals will have to choose between different temperatures, meaning there will always have to be an intermediate temperature that individuals will use to traverse.

I believe many aspects of this equipment make it unique (see Appendix B Supplementary Material 6 for a comparison table). This includes, but is not limited to, its ability to be set to either a constant or dynamic temperature regime, as well as being grouped together to create various thermal landscapes. For the trials here, I created the ATAs to have 18 temperature points formatted in a single row, but users can create a different thermal landscape appropriate to their needs. For example, users could group individual ATAs together to create a resource-grid, where temperature points are both adjacent and parallel to each other (Fig. 3.6).

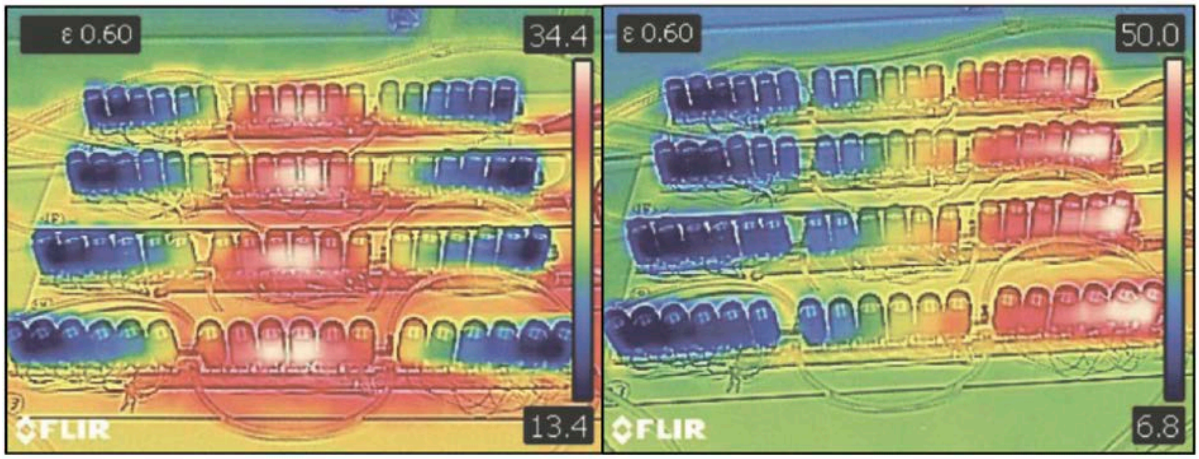

Figure 3.6: Two examples of how ATAs can be grouped together to create "resource-grid" landscapes, captured with a thermal imaging camera to demonstrate the variance in temperatures along the ATAs.

Another important aspect of this equipment is the option of using cages rather than restricting individuals to specific temperature points. The cages allow animals to access all temperature points and interact with other individuals in the cage while choosing resources, providing users with information on thermal behaviour. Although it is widely-recognized that behaviour needs to be incorporated into models predicting effects of climate change (e.g., Sunday et al., 2014), thermal preference and thermoregulatory behaviour in small ectotherms is difficult to measure, in large part due to the lack of practical equipment that provides both sufficient temperature resolution and scale (Rajpurohit & Schmidt, 2016).

ATAs coupled with cages also allow users to gain information on species interactions and evolutionary responses. Users can explore multiple types of species interactions (e.g., predation, competition, etc.) between two or more species in a thermal environment, as well as conduct multi-generation experiments to see how these interactions affect adaptive potential to thermal environments. Failing to incorporate these processes into model predications is a major limitation to many global change studies (Guisan & Thuiller, 2005; Dormann, 2007; Kearney & Porter, 2009; Hoffmann & Sgrò, 2011). Experiments using ATAs will compliment field data and modelling to better resolve and predict the limits and evolutionary potential of organisms vulnerable to climate change.

3.6 Highlights

- I designed an adjustable temperature array that can be used to answer broad ecological and evolutionary questions on small flora and fauna.
- I validated the equipment using four relevant thermal landscapes that may be broadly applicable to researchers.
- This equipment provides a completely customisable resource for researchers in thermal biology and ecology.

Chapter 4: Temperature preference and thermal fitness are tightly coadapted in both a generalist and a specialist species of *Drosophila*

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Contributions: JC, CH, and MH conceived the experiment. JC conducted the experiment and collected data. JC analysed data with important input from MH and CH. JC wrote the manuscript with important contributions from MH and CH.

4.1 Abstract

Ectotherms use behaviour to reduce exposure to lethal environmental temperatures and to achieve an optimal body temperature for performance. This is theorized to cause a coupling of thermoregulatory behaviour and thermal performance in what is known as the 'thermal coadaptation hypothesis'. Likewise, oviposition temperature preference should correlate to performance because females should prefer environments that support optimal development of their offspring. Here, I examined two competing hypotheses for the thermal coadaptation hypothesis in a thermal generalist (*Drosophila serrata*) and a thermal specialist (*Drosophila birchii*) species of *Drosophila* by examining oviposition temperature preference and thermal performance in productivity, development speed, and wing size. Thermal generalist and thermal specialist species exhibit different thermal performance strategies, so coadaptation between temperature preference and performance may also differ. Productivity in both the generalist and the specialist species were tightly coadapted to oviposition temperature preference. I found no evidence of coadaptation in development speed and body size. This study is the first to provide support for the thermal coadaptation hypothesis between oviposition preference site and fitness. This is important for examining whether thermoregulatory behaviour can aid or hinder adaptation to thermal environments. However, additional research is needed in more species to fully understand whether thermal coadaptation exists between oviposition temperature preference and fitness.

4.2 Introduction

Temperature is an important abiotic factor affecting species distribution (Cossins & Bowler, 1987). This is particularly true for small ectotherms because of their limited ability to physiologically thermoregulate (Stevenson, 1985; Parmesan et al., 2000; Dillon et al., 2009; Sillero et al., 2014; Isaak et al., 2017). The thermal environment where they live is therefore largely determined by physiological and behavioural traits, and is referred to as the thermal niche (Magnuson et al., 1979). Understanding the thermal niche of species, and the complexity of factors contributing to it, is increasingly important in regards to climate change research. Only by understanding the details of the thermal niche can we accurately assess the current and potential impact of climate change on species and communities.

The contributions of physiological and behavioural traits to the thermal niche are estimated by different methods: physiological traits are commonly measured as *performance* along a thermal gradient, whereas thermoregulatory behaviour is controlled by and measured as *temperature preference* along a thermal gradient (Angilletta et al., 2002). In the wild, many species use behaviour to maintain their body temperature at temperatures where optimal performance occurs and to reduce localized effects of spatial and temporal variation (Angilletta et al., 2002). Theoretically, it then makes sense that temperature preferences should coevolve with optimal performance (Cowles & Bogert, 1944; Beitinger & Fitzpatrick, 1979; Coutant, 1987; Gilchrist, 1995; Angilletta et al., 2002; Martin & Huey, 2008; Dillon et al., 2009). An evolutionary shift in optimal performance temperature should apply selective pressure for individuals who prefer a similar temperature because this directly results in increased performance and hence higher fitness. For example, individuals avoiding extreme conditions and moving towards conditions optimal for performance (e.g., by moving to the sun or shade; Isaak et al., 2017) will drive selection of temperature preference towards temperatures where peak fitness occurs (Huey & Kingsolver, 1989; Angilletta et al., 2002; Angilletta, 2009; Halliday & Blouin-Demers, 2015). This coupling of optimal performance temperature and temperature preference is often referred to as the ‘thermal coadaptation hypothesis’.

The thermal coadaptation hypothesis is tested by quantifying thermal performance as a curve across relevant temperatures for each trait of interest, and then comparing the optimal performance temperature (T_{opt}) to the average temperature preference of adults (T_{pref}). Thermal performance curves (TPCs) measure performance along a thermal gradient, which generally shows performance increasing slowly towards an optimal temperature before

decreasing rapidly towards a maximum thermal limit where fitness reaches zero (Fig. 4.1A). Thermal performance curves can be used to examine the thermal range a species can tolerate and the range the species performs best within. These curves are often used to compare thermal generalist versus thermal specialist species. Thermal generalists should exhibit a wider TPC than thermal specialists because they use a broader range of thermal environments (Gilchrist, 1995). Further, it is hypothesized that a trade-off exists between generalists and specialists, whereby a generalist has a lower peak fitness value (P_{max}) at T_{opt} than a specialist (Gabriel & Lynch, 1992; Gilchrist, 1995; Palaima, 2007; Condon et al., 2015; Fig. 4.1A). In this sense, generalists are often referred to as a 'jack of all trades (i.e., temperatures), but master of none' (Huey & Hertz, 1983). On the other hand, specialists would exhibit a greater fitness than generalists at their T_{opt} but a trade-off would occur at the niche edges (Fig. 4.1A; for examples, see Gilchrist, 1995; Blouin-Demers et al., 2003; Angilletta, 2009; Phillips et al., 2014).

Table 4.1: List of abbreviations used and descriptions of measurement. Alternative abbreviations found throughout the literature are also listed.

Abbreviation	Term	Description of measurement	Alternative abbreviations used
B_{80}	TPC breadth at 80% P_{max}	Temperature range where performance is above 80% P_{max}	T_b, T_{br}, P_{br}, P_{80}
P_{max}	Maximum performance	Maximum performance value	U_{max}, R_{max}
T_{opt}	Thermal optimum	Temperature where P_{max} occurs	T_o
T_{pref}	Temperature preference	Average temperature preference	T_p
T_{set}	Temperature preference range	80^{th}-100^{th} quantiles of temperature preference range; (upper 20% of preferred temperatures)	$T_{set(80)}$
TPC	Thermal performance curve	Curve describing performance/fitness over a thermal gradient	

The thermal coadaptation hypothesis is straightforward in theory, but it becomes more complex when considering the different thermal performance strategies exhibited by generalist and specialist species. For example, a thermal generalist is sometimes referred to as a thermoconformer because their body temperature conforms to the environmental

temperature and is physiologically able to perform within a wider range of temperatures than thermal specialists, which are often referred to as more specific thermoregulators because they more-readily use behaviour to remain in a narrower temperature range (Heinrich, 1981). Specific evidence on whether temperature preference is more tightly coevolved with thermal performance in generalists or specialists is currently lacking (but see Blouin-Demers et al., 2003 and Buckley et al., 2015 for important insights into how thermal physiology relates to thermal behaviour in thermoconformers and thermoregulators). Here, I examine two contradicting hypotheses for the thermal coadaptation hypothesis in a thermal generalist and a thermal specialist species.

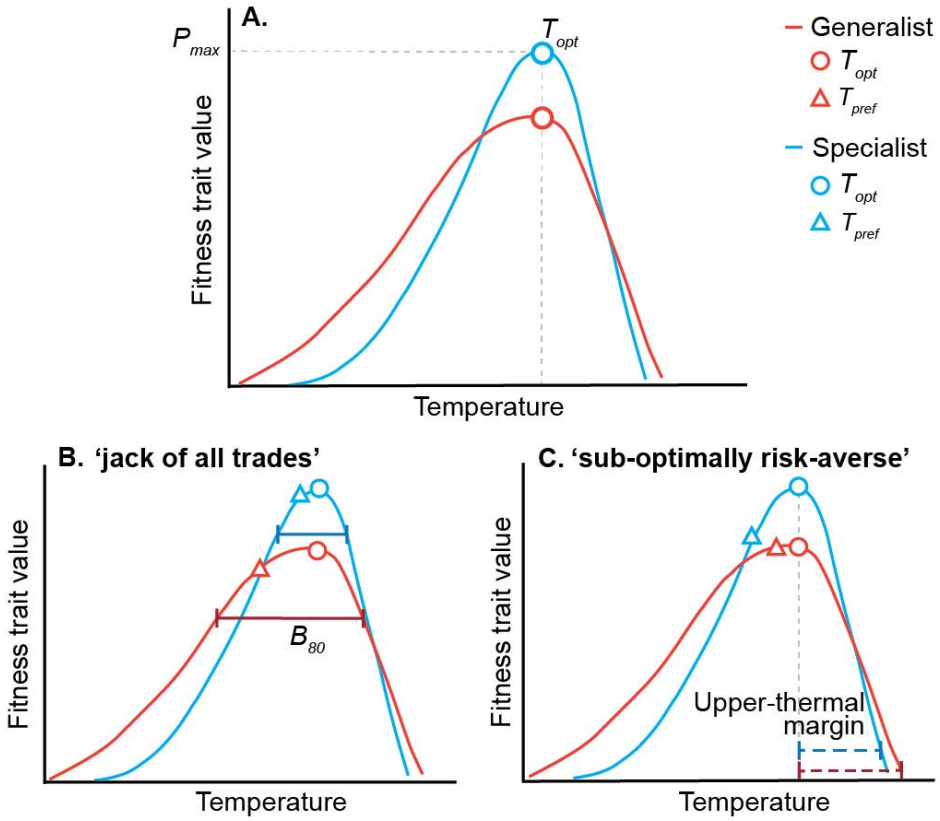

Figure 4.1: Temperature preference should coadapt with optimal fitness within a thermal generalist and a thermal specialist species.

(**A**) TPCs for a thermal generalist (red) versus a thermal specialist (blue) species. Generalists should exhibit a wider thermal niche than a specialist, while specialists often have a higher peak fitness value (P_{max}) at their optimal temperature (T_{opt}) caused by a trade-off at the niche edges. (**B**–**C**) There are two contradicting hypotheses predicting whether temperature preference (T_{pref}) should coadapt more perfectly to thermal performance in a generalist or in a specialist species. The first hypothesis (**B**) predicts that coadaptation should occur over a wider range in generalists because there is less of a fitness trade-off associated with a temperature preference further away from T_{opt} in a generalist species (considered a 'jack of all trades') than in a specialist species. This is reflected by a wider peak performance range (B_{80}) in a generalist than in a specialist. If this hypothesis is correct, then T_{pref} and T_{opt} would be more perfectly coadapted in specialists than in generalists. The second hypothesis (**C**) predicts that T_{pref} should occur at a wider, and more suboptimal, gap from T_{opt} in specialists because specialists have a narrower 'upper thermal margin'—meaning there is a higher-risk associated with preferring a temperature closer to the lethal upper thermal limits in specialists than within generalists. If this hypothesis is correct, then T_{pref} and T_{opt} would be more perfectly coadapted in generalists than in specialists.

The first hypothesis is well-recognized, and assumes that there is less selective pressure for coadaptation between peak fitness and temperature preference in generalists (Blouin-Demers

et al., 2003). This is because there is relatively less advantage to being exactly at T_{opt} in generalists than in specialists because of the broad peak of a generalist TPC versus the narrow peak of a specialist TPC (also known as peak performance breadth; B_{80}; Fig. 4.1B). Individuals may exhibit preference for a broader range of thermal environments at the centre of the thermal niche where being away from T_{opt} results in only a slightly lower fitness and hence coadaptation between T_{opt} and T_{pref} may be looser (i.e., 'jack of all trades' hypothesis; Fig. 4.1B). In contrast, specialists should exhibit temperature preference that mimics their TPC; i.e., a narrower breadth (Fig. 4.1B) because there are serious fitness consequences to any small variation away from T_{opt}.

The second hypothesis is from a theoretical model considered by Martin and Huey (2008)—whose models predict that preferred body temperatures should occur at slightly lower temperatures than peak fitness temperature (Fig. 4.1). They explain the rationale for their hypothesis by first noting that ectotherm TPCs are often asymmetric and exhibit a rapid decline in fitness after T_{opt}; and second, that ectotherms cannot thermoregulate perfectly (as a result of not being able to physiologically thermoregulate) and so they most likely experience a range of body temperatures. As such, a so-called 'safety-net' may occur for ectotherms, where T_{pref} occurs at suboptimal temperatures because fitness above T_{opt} decreases much more rapidly and may be fatal at a temperature slightly higher than T_{opt} but not below. The range between T_{opt} and the upper thermal limit where fatality occurs is referred to here as the 'upper-thermal margin' (Fig. 4.1C).

When looking at this hypothesis in relation to thermal specialists and thermal generalists, Martin and Huey (2008) theorize that the gap between preferred body temperature and optimal body temperature should be larger in specialists than in generalists (Fig. 4.1C). This is because specialists exhibit a narrower TPC, so even a slight increase in temperature will be more detrimental to fitness than the same shift in a generalist (Martin & Huey, 2008). Hence, a T_{pref} that occurs further below T_{opt} in the specialist (which has a narrower 'upper-thermal margin') decreases this risk while this is much less of a risk in a generalist (due to their wider 'upper-thermal margin'; Fig. 4.1C). In this sense, it is better for a specialist to be 'sub-optimally risk-averse' than to hazard a T_{pref} that is tightly coadapted to T_{opt} (i.e., 'sub-optimally risk-averse' hypothesis; see Fig. 4.1C). This prediction is in direct contrast to the 'jack of all trades' hypothesis (Fig. 4.1B).

Empirically, the majority of research on the 'thermal coadaptation hypothesis' has focused on correlating adult performance traits in large ectotherms to adult thermal preference, and these studies have found mixed results (reviews in Angilletta et al., 2002;

Halliday & Blouin-Demers, 2015). In lizards, some studies have found a significant relationship between T_{opt} and T_{pref} for sprint speed (Bennett, 1980; Huey & Bennett, 1987; Bauwens et al., 1995; Gaby et al., 2011; Buckley et al. 2015), while others have not (Huey & Kingsolver, 1989; McElroy, 2014). In juvenile frogs, there has been evidence for coadaptation between temperature preference range (commonly called T_{set}) and the optimal temperature for locomotor performance (Sanabria et al., 2013). In turtles, T_{opt} for swimming and righting locomotor performance fell within the T_{set} for preferred basking temperature (Ben-Ezra et al., 2008). In snakes, T_{opt} for tongue-flicking, striking, and swimming performance were similar to T_{set} in one species but higher than T_{set} in another species (Blouin-Demers et al., 2003). In both fish and snakes, the plasticity of T_{pref} has been shown to correlate to T_{opt}. In fish, a higher temperature maximizes growth when food intake is high and fish that ingested more food were found to prefer a higher temperature than those that did not (Mac, 1985). However, the opposite was subsequently found in a different species of fish (Morgan & Metcalfe, 2001). In snakes, the preferred temperature differed based on food consumption with those that had recently consumed a large meal preferring a higher temperature than fasted snakes, the former of which correlated to T_{opt} for digestion (Dorcas et al., 1997).

These above studies use indirect measures of fitness and do not empirically test whether ultimate fitness (i.e., directly related to reproductive output) corresponds to temperature preference. The studies that test ultimate fitness in relation to temperature preference are limited to two studies using *Tribolium* (i.e., flour beetles), which are built upon each other and use the same temperature preference data (Halliday & Blouin-Demers, 2015; Halliday & Blouin-Demers, 2017), and one study using *Caenorhabditis elegans* (Anderson et al., 2011). These studies have provided a basis of literature testing the thermal coadaptation hypothesis in terms of ultimate fitness but have found mixed support for it. This could be because the studies used eight or less thermal environments to test physiological tolerances of fitness traits, leading to coarse estimates of T_{opt} that may not be as accurate as estimations from TPCs defined over finer temperature gradients. Another consideration is that temperature preference was measured by categorizing the temperature preference of adult *Tribolium* and adult *C. elegans* after they have moved along a thermal gradient and 'chosen' their thermal environment. This is important when considering adults are the only life-stage in many ectotherms that are able to behaviourally regulate and move to preferred temperatures (eggs are sessile and larvae may have limited mobility). However, these studies

did not consider how adult temperature preference influenced the development of offspring through breeding site or oviposition site preference.

Females may prefer certain thermal environments over others to deposit eggs to increase fitness, but oviposition site preference is a vastly understudied trait in thermal biology (Dillon et al., 2009). If animals are given a choice of breeding resources along a thermal gradient then this would allow for both female adult temperature preference and oviposition site preference to be measured. For example, females may prefer to oviposit at higher temperatures where development has been shown to be more rapid, which would benefit offspring by allowing them to use available resources before later-emerged individuals (Dillon et al., 2009). This would also inherently incorporate adult temperature preference (Dillon et al., 2009) and allow for measurement of offspring ultimate fitness traits as a way of comprehensively describing temperature preference.

Here, I measured temperature preference and TPCs of a closely-related generalist *Drosophila* and specialist *Drosophila* fly. I present a study that is novel for three reasons. First, I incorporated oviposition preference into temperature preference and compared it to the TPCs. Second, I investigated whether a generalist's temperature preference range is wider than a specialist's for *ultimate* fitness traits (productivity and development speed), which are thought to be more appropriate predictors of the thermal coadaptation hypothesis than proximate fitness traits (Halliday & Blouin-Demers, 2015; Halliday & Blouin-Demers, 2017). To my knowledge, this is the first study to present the two competing hypotheses ('jack of all trades' verse 'sub-optimally risk-averse') for the thermal coadaptation hypothesis in a generalist and a specialist species, and to test them in an empirical example. I also measured one proximate trait (i.e., a trait indirectly affecting reproductive success; wing size) and compared it to temperature preference. Third, I examined the previous two aims by measuring TPCs of productivity, development speed, and wing size over a fine temperature scale using 17 thermal environments.

4.3 Methods

Study system

I used two sister-species of *Drosophila* from the *montium* subgroup found along the east coast of Australia. *Drosophila serrata* is generalist species found from lowland sclerophyll woodland and up to mountain-top rainforests (Schiffer et al., 2004). *Drosophila birchii* is

considered a rainforest specialist and, in the Australian Wet Tropics region (Schiffer et al., 2004), is only found in mid and high elevation rainforest. The two form a generalist/specialist pair that are a well-studied system for adaptation due to differences in their physiology, distribution, and habitat preferences (Kelemen & Moritz, 1999).

I collected wild flies from two populations of each species, both of which were allopatric to the other species. I collected *D. serrata* flies from two areas around Rockhampton, QLD (Raglan: 23°42'49.74"S, 150°49'0.10"E; Granite Creek: 24°36'47.25"S, 151°40'10.45"E); and I collected *D. birchii* flies from Paluma National Park (19° 0'16.27"S, 146°12'35.59"E) and Mt Lewis National Park (16°35'30.36"S, 145°16'27.78"E). I used two populations per species to provide population-level replication within species. I collected flies using banana baits (as described in Higgie and Blows, 2008), identified them to the species level, and created isofemale lines for each species by placing one gravid female in a breeding vial and then allowing only her offspring to breed. Isofemale lines maintain the underlying genetics of the wild-caught female within her progeny (Hoffmann & Parsons, 1988a; David et al., 2005). Isofemale lines were maintained in a controlled environment room at 23°C in 12 hr light:dark cycles for approximately 18 generations before being bred to create stock mass bred populations. Mass bred populations were created by breeding the offspring of ten isofemale lines from each wild population. Mass bred populations were maintained at large numbers ($N > 1000$) for approximately one year before the start of this experiment.

Thermal Performance Curves and Temperature Preference measurements

Thermal performance (i.e., TPC) and temperature preference treatments were measured for each population across four blocks in a complete randomized block design ($N = 4$ per population per each treatment). This allowed for eight replicates per species where both treatments (TPC and temperature preference) for each of the populations were measured in each block. Thermal performance curves and temperature preferences were measured along a thermal gradient from 20°C–36°C in 1°C steps. Linear thermal gradients were set up using temperature arrays (Fig. 4.2) as described in Cocciardi et al. (2019). Temperature arrays were set-up across two controlled temperature rooms and were randomly assigned replicates at the start of each block, but both treatments for a single population were placed within the same experimental room to reduce variation. 10 mL of *Drosophila* feeding and breeding material were placed at every temperature point (1°C) on the temperature array, and 10 µg of a diluted

water:baker yeast solution (10:1 parts) was deposited on top to promote ovipositing. This created a thermal resource gradient where adult temperature preferences, oviposition temperature preferences, and fitness could be measured. All food vials were randomized across temperature arrays. The directionality of the thermal gradient was also randomized across temperature arrays. Each block ran for 17 days to allow for full development of all potential offspring. Temperature was recorded at each temperature point every minute for the duration of the experiment and monitored throughout the experiment. The mean temperature deviation at temperature points across the entirety of the experiment was 0.16°C ± 0.05°C (that is, thermal environments were 0.16°C warmer on average than their setpoint).

Density-controlled bottles were created one generation before the start of the experiment to limit larval competition and control for individual body size of the offspring that were subsequently used in the experiment. Three density-controlled mass bred bottles per population and species were created by breeding 30 males and 30 females collected at random from each stock mass bred population. Flies were placed in a 300 mL bottle, containing 100 mL of *Drosophila* feeding and breeding material, and left for 48 hrs to breed before being removed. Virgin flies were subsequently collected from the offspring of the mass bred bottles less than 12 hrs after they eclosed, sexed under light CO_2 anaesthetic, and randomly placed in holding vials that contained five individuals of the same sex from their population. Flies were then left to recover from the anaesthesia for 3–4 days before the experiment started.

Thermal performance was measured by placing one female and two males into each vial at the temperature points and stoppering the vial with a porous plug to confine individuals to each temperature point (Fig. 4.2A). Two males were placed in each vial to increase the chances of mating. Female *D. serrata* have been shown to enact post-copulatory sexual selection to their first mate (Frentiu & Chenoweth, 2008; Collet & Blows, 2014) and *D. birchii* have limited sperm storage from a lack of spermathecae (Saxon et al., 2018a). Therefore, there is very limited possibility of increased progeny due to multiple mating and hence this was not taken into account. In contrast to thermal performance, in temperature preference treatments, flies were not confined to each temperature point (Fig. 4.2B). Instead, an enclosure was placed around the temperature array and individuals were able to move freely to choose thermal resources. The enclosures were built using a 34 L plastic container and mesh (methods described in Cocciardi et al. 2019) and two 5 L glass containers filled with water and covered with a sponge were placed along either end of gradient to reduce

dehydration. At the start of the experiment, 17 females and 17 males were placed in the cage at the centre of the gradient and allowed to disperse to thermal resources for 48 hrs before being removed. 34 individuals were placed in the cage to represent the same number of mating pairs as in the thermal performance treatment.

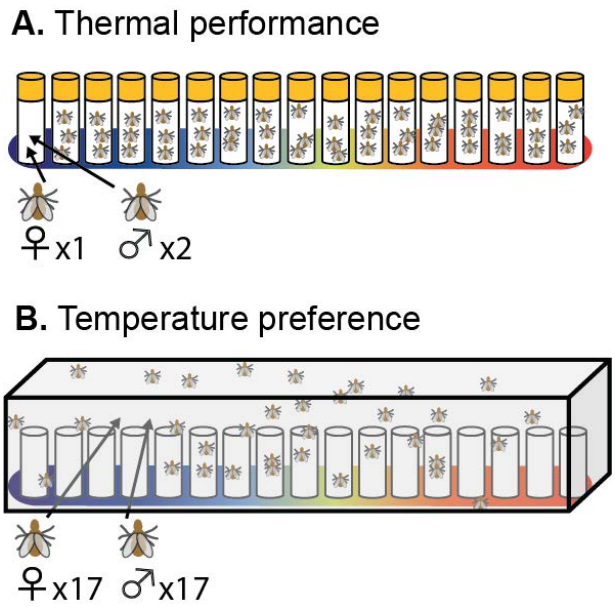

Figure 4.2: Experimental design to measure thermal performance and temperature preference in two species of closely-related Drosophila.
(**A**) Flies were confined to thermal resource vials to measure thermal performance curves (TPC) in productivity, development speed, and wing size across a linear thermal gradient of 20°C–36°C in 1°C steps. (**B**) Flies were placed in a cage with un-stoppered thermal resource vials and were able to choose their preferred thermal environment and thermal-ovipositing resource, allowing for a measure of thermal preference inclusive of oviposition preference.

Parental survival was checked every 12 hrs for 48 hrs at which time all parents were removed. Pupation cards were placed within vials at 72 hrs. Vials were then checked every 24 hrs for new emergences and all new emergences were collected, sexed, and counted per 24 hr period for a total of 15 days after parents were removed. A random subset of female flies from each emergence period was frozen and used for wing size measurements. In addition, ten female parents per treatment were randomly selected after mating and their left wings were mounted, photographed, and digitized to obtain average parent wing size (using methods described to obtain wing size measurements below). This, in addition to rearing

parents in density-controlled mass breds, was done to confirm no further variation was introduced from parent body size (R^2 = 0.005, $F_{2,192}$ = 1.54, P = 0.218).

Fitness measures

Productivity, development speed, and wing size were assessed at each temperature point to create TPCs for ultimate fitness traits. Productivity was used to calculated temperature preference within preference treatments because it incorporates both adult temperature preference and female oviposition preference. Productivity was counted as the total number of offspring that emerged per vial. Average development speed was measured as the inverse of the average 24 hr period that adults emerged from each vial. Body size was obtained from three random female offspring per temperature point and was measured as wing size.

Wing size was measured by digitizing and measuring the left wing of three females per temperature and treatment. To do so, a random subset of females from each emergence day at each temperature point was frozen. At the end of the experiment, three females per temperature point were randomly selected from all emergences and the left wings were mounted on a microscope slide using fine forceps and double-sided tape. Photos of wings were taken using a Leica Image microscope (*LASV3.8*) and images were randomized and collated as a TPS file using *tpsUtil* (Rohlf, 2010b). Ten landmarks were placed on consistent morphometric wing features using the program *tpsDig2* (Rohlf, 2016) (as defined in Chapter 2). Outliers and landmarking errors were identified and removed using *tpsRelW* (Rohlf, 2010a) before wing measurements were computed.

Landmarked coordinates underwent a Generalised Procrustes Analysis (GPA) superimposition (Rohlf & Slice, 1990), where wings are aligned on top of each other by superimposing images upon one another over an average configuration. The centroid size for each wing is then calculated from the square root of the summed squared distance between centroid configuration and landmarks. This provides a measure of overall size (Rohlf & Slice, 1990).

Statistical analyses

All data was analysed in R (R Core Team, 2019) and data exploration was carried out prior to analyses using the protocols set out in Zuur and Ieno (2016). TPCs were created by fitting pre-defined functions (Appendix C Table 1) for each species and population using non-linear least squares (nls). The *rTPC* (Padfield & O'Sullivan, 2020) and *nls.multstart* package

(Padfield & Matheson, 2020) in R were used to fit curves. Model fits were weighted by the inverse of the variance for productivity due to heteroscedasticity of variance across temperatures. Standard un-weighted models were used for development speed and wing size because of homoscedasticity and because there were uneven sample sizes across temperature points. The best fit curve was chosen as the function that produced the best AICc score (Appendix C Tables 2–4; Appendix C Figures 1–3). I used AICc because it corrects the Akaike Information Criterion (AIC) for small sample sizes and is recommended as the standard value to use for model selection instead of BIC (which may select a too-simplistic model for real-world data; Burnham & Anderson, 2004; Brewer et al., 2016). If AICc scores were less than 2 from the best fit value, both models were weighted and parameters were estimated by model averaging (Burnham & Anderson, 2004; Appendix C Figures 1-3). 95% confidence intervals were calculated for each curve by using first-order Taylor expansion and Monte Carlo simulation (K = 100,000) using the function 'predictNLS' in the R-package *propagate* (Spiess, 2018).

TPC parameters were estimated directly from the function. TPC parameters included the thermal optimum (T_{opt}), the trait value representing peak performance (P_{max}), and the breadth of the curve where performance is above 80% peak performance (B_{80}, an indicator of specialization). B_{80} was compared between *D. serrata* and *D. birchii* using a one-sided t-test to investigate whether the generalist (*D. serrata*) used in this study has a significantly wider thermal niche than the specialist (*D. birchii*), and P_{max} was also compared using a one-sided t-test to investigate whether the specialist exhibits a higher P_{max} than the generalist.

Oviposition temperature preference (T_{pref}) was calculated as the T_{opt} from the best fit curve for productivity within the temperature preference treatments (Appendix C Table 5 and Appendix C Figure 4). T_{opt} was regressed upon T_{pref} for each trait to obtain an index of coadaptation between optimal and preferred temperatures (Huey & Bennett, 1987), and the parameters were compared using a Pearson correlation to investigate whether a relationship exists. In addition, the 80th percentile (i.e., upper 20%) of preferred temperatures, known as a 'set point' range for thermal preference (T_{set}; Hertz et al., 1993), was also calculated and I examined whether T_{opt} fell within each T_{set} range. 95% confidence intervals for T_{pref} and T_{set} ranges were calculated and compared to the 95% confidence intervals of T_{opt}.

To test whether oviposition temperature preference coadapted less directly with thermal performance in the generalist species (the 'jack of all trades' hypothesis) or in the specialist species (the 'sub-optimally risk-averse' hypothesis); T_{pref} was subtracted from T_{opt} for each population and trait, and values were compared between species using a one-sided t-

test in both directions. In addition, B_{80} for temperature preference was directly estimated from the best fit curve for the temperature preference treatment and compared between species using a one-sided t-test to investigate whether the generalist exhibited a wider peak temperature preference range than the specialist ('jack of all trades' hypothesis).

4.4 Results

Thermal Performance Curves

TPCs were modelled using four replicates per population (N = 8 per species). Development speed and wing-size sample sizes varied across the temperature gradient depending on whether offspring developed at each temperature or not. In addition, temperature preference for the *D. birchii* Mt Lewis population was obtained from only three replicates due to equipment malfunctioning.

The thermal optimum for productivity was located 2.9°C higher in *D. serrata* than in *D. birchii*, but this was not a significant difference ($t_{1.0}$ = -1.57, P = 0.361). Consistent with theory predicting a trade-off between niche breadth and peak fitness in generalists (Fig. 4.1), the generalist *D. serrata* was found to have a wider B_{80} than in the specialist *D. birchii* ($t_{1.8}$ = -4.51, P = 0.028). In contrast, the generalist *D. serrata* did not have a lower productivity P_{max} than in the specialist *D. birchii* ($t_{1.2}$ = -2.66, P = 0.903; Table 4.2 and Fig. 4.3A).

Table 4.2: Thermal performance curve parameters for *D. serrata* and *D. birchii* for each ultimate fitness trait measured. Means and standard errors are noted for T_{opt}, P_{max}, and B_{80} (N = 2).

Trait	Species	T_{opt} (°C)	P_{max}	B_{80} (°C)
Productivity	*D. birchii*	23.63 ± 0.03	62.21 ± 3.03	3.94 ± 0.71
	D. serrata	26.54 ± 2.62	81.01 ± 9.50	7.85 ± 1.00
Development speed	*D. birchii*	27.75 ± 1.41	0.119 ± 0.009	8.14 ± 2.33
	D. serrata	26.96 ± 0.45	0.111 ± 0.001	10.30 ± 0.34
Wing size	*D. birchii*	20.77 ± 1.09	936.27 ± 8.69	11.5 ± 2.12
	D. serrata	20.00 ± 0.00	977.46 ± 4.57	12.0 ± 1.41

The thermal optimum for development speed was similar between species (T_{opt}: t_2 = 0.75, P = 0.531; Table 4.2). Maximum development speed was also very similar between *D. serrata* and *D. birchii* (P_{max}: t_2 = 1.26, P = 0.167), with the exception of one population (Mt Lewis)

of *D. birchii* exhibiting a quicker maximum development rate (P_{max}: 0.126 days^{-1}) and at a higher optimal temperature (T_{opt}: 28.74°C) than the other populations (Table 4.2 and Fig. 4.3B). Peak performance breadth of TPCs for development speed were not significantly different between species (B_{80}: t_2 = -1.29, P = 0.162; Table 4.2).

The thermal optimums were similar for both species for wing size (T_{opt}: t_1 = 1, P = 0.500; Table 4.2 and Fig. 4.3C). *Drosophila birchii* exhibited a smaller maximum wing size than *D. serrata* (P_{max}: $t_{1.5}$ = -5.93, P = 0.025). This is most likely due to *D. birchii* parents also being smaller (Parental generation: *D. birchii* = 941.2 ± 22.3 SD; *D. serrata* = 962.9 ± 38.8 SD) and reflecting how *D. birchii* are generally a smaller species than *D. serrata* (Ayala, 1965). Peak performance breadths were not significantly different between species for wing size (B_{80}: $t_{1.74}$ = -0.28, P = 0.405; Table 4.2 and Fig. 4.3C).

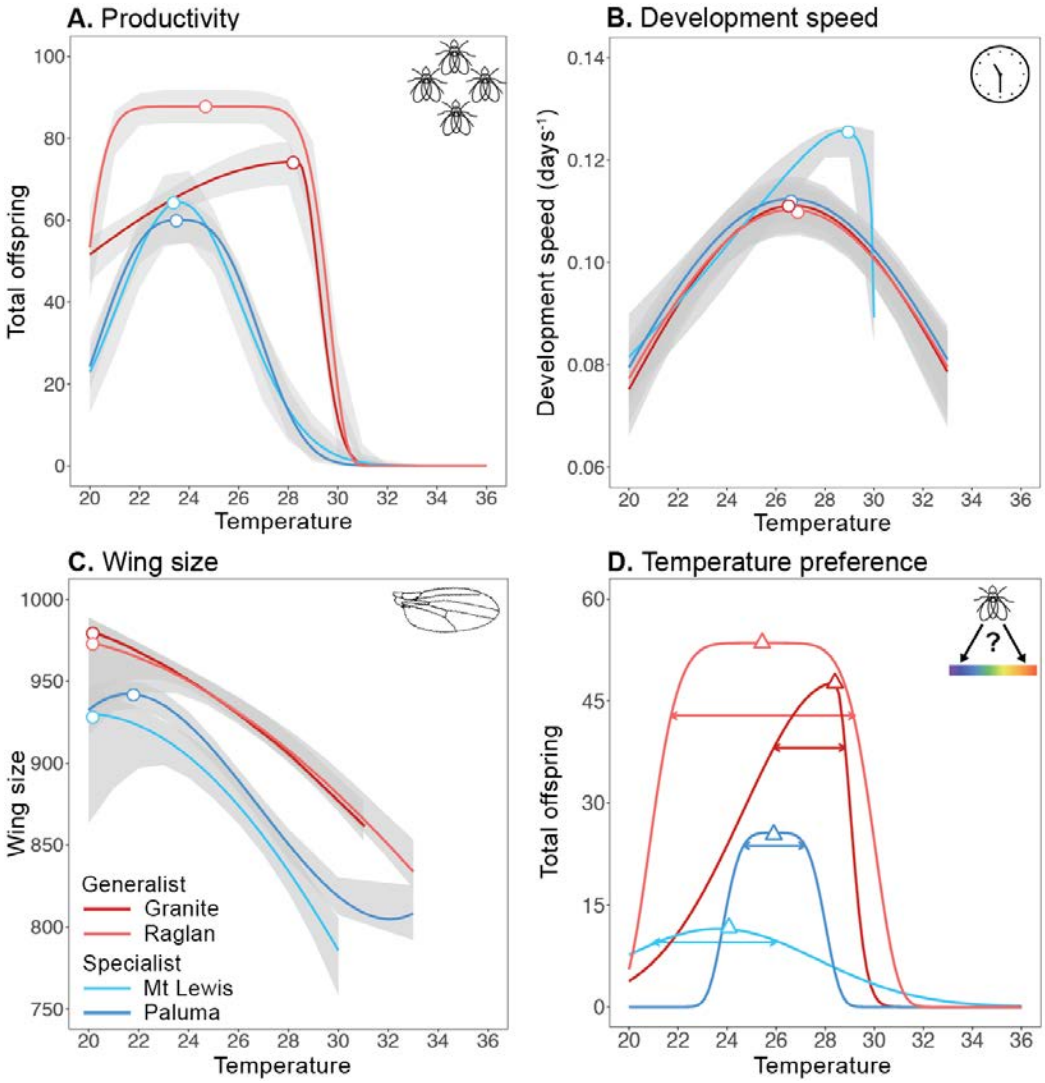

Figure 4.3: Thermal performance curves for three fitness traits and temperature preference curves for a thermal generalist (red) and a thermal specialist (blue) *Drosophila* species. Thermal performance curves for each population for (**A**) productivity, (**B**) development speed, and (**C**) wing size for a thermal generalist and a thermal specialist species. The generalist *Drosophila serrata* populations are shown in dark red (Granite) and light red (Raglan) and the specialist *D. birchii* populations are shown in dark blue (Paluma) and light blue (Mt Lewis). T_{opt} for each curve is indicated by a circle and 95% confidence intervals for curves are shown with grey shading. (**D**) Temperature preference was measured as total offspring that emerged after adults chose where to oviposit. T_{pref} for each curve is indicated by a triangle and B_{80} for temperature preference is indicated by the horizontal line.

Oviposition Temperature Preference

Drosophila birchii exhibited a cooler, but not significantly different, oviposition temperature preference than *D. serrata* (*D. birchii*: 24.71°C ± 0.90 SD, *D. serrata*: 26.91°C ± 2.09 SD; t-test on T_{pref}: $t_{1.36} = -1.36$, $P = 0.178$; Fig. 4.3D). *Drosophila birchii* exhibited an optimal temperature preference breadth (B_{80} for temperature preference) that differed by only 1.54°C from *D. serrata* and this was not significantly different from *D. serrata* (*D. birchii*: 3.35°C ± 1.51 SD, *D. serrata*: 5.19°C ± 3.18 SD; t-test on B_{80}: $t_{1.4} = -0.62$, $P = 0.309$; Fig. 4.3D).

Comparing Oviposition Temperature Preferences to Thermal Performance Curves

The thermal optimum for productivity is strongly correlated to oviposition temperature preference ($r = 0.959$, $P = 0.040$) and I found evidence for significant coadaptation between the two ($R^2 = 0.92$, $F_{1,2} = 23.27$, $P = 0.040$). The thermal optimum for productivity (T_{opt}) fell within 1°C of T_{pref} for *D. serrata*, and within 1.7°C of T_{pref} for *D. birchii*, and within all T_{pref} confidence intervals except for the Paluma population of *D. birchii* (Fig. 4.4). However, the thermal optimum for Paluma still fell within the confidence interval range for T_{set} (Fig 4.4D). T_{pref} was also located within each B_{80} range for all populations of both species. This indicates that temperature preference fell within the temperature ranges where fitness was optimal (i.e., 80% of peak performance) for both species.

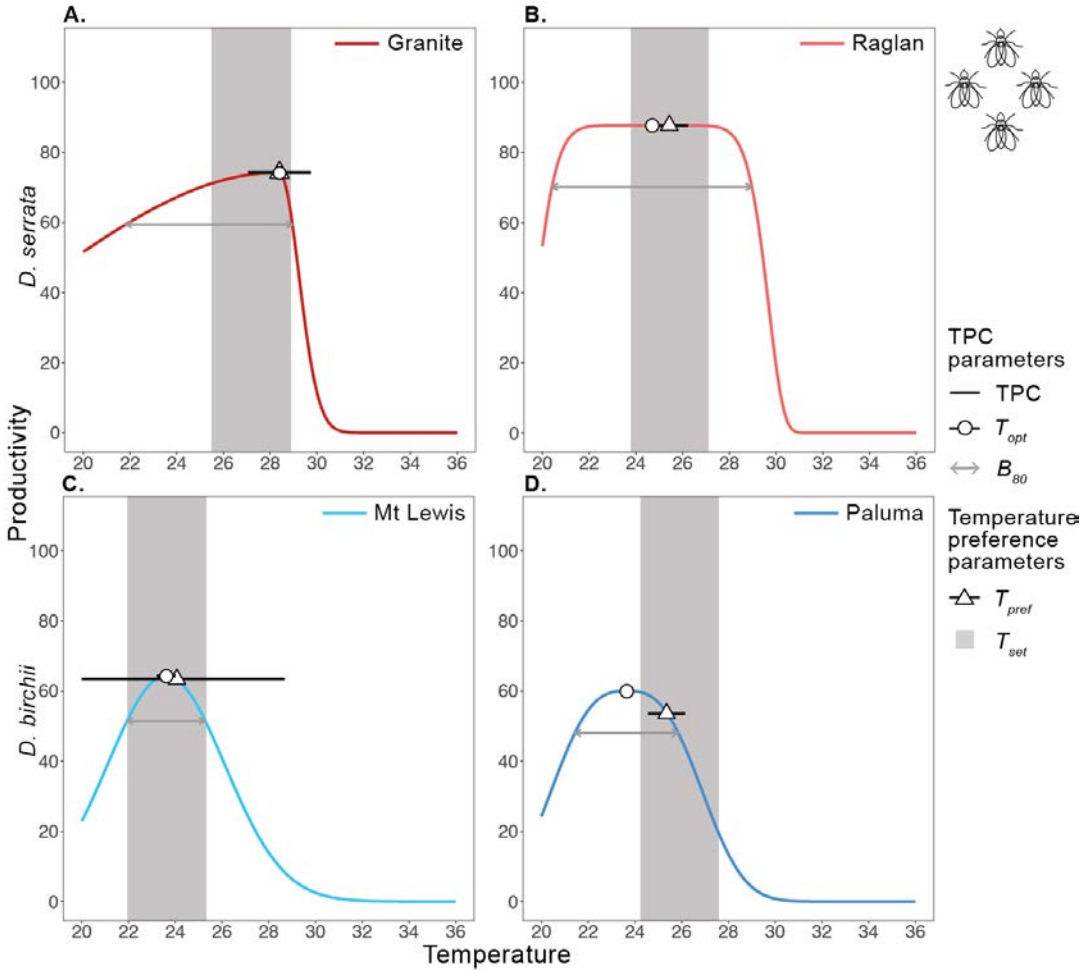

Figure 4.4: Comparison of productivity TPCs and temperature preference of a thermal generalist (red) and a thermal specialist (blue) *Drosophila* species.

Productivity TPCs for populations of the generalist *D. serrata* are shown in red (**A**, **B**) and the specialist *D. birchii* are shown in blue (**C**, **D**). TPC parameters shown include T_{opt} and B_{80}. Temperature preference parameters shown include where T_{pref} falls along the TPC and the 80% temperature preference range (T_{set}). 95% confidence intervals for T_{opt} and T_{pref} are shown as black error lines.

Overall, the thermal optimum for development speed does not correlate to oviposition temperature preference (r = -0.384, P = 0.615), and I did not find evidence for significant coadaptation between the two (R^2 = 0.15, $F_{1,2}$ = 0.35, P = 0.615). This result was driven by the *D. birchii* Mt Lewis population (Fig. 4.5C). Nevertheless, T_{opt} and T_{pref} for development speed were found to have overlapping 95% confidence intervals for all populations (Fig. 4.5), indicating the two parameters are not significantly different from one another. T_{opt} fell within 1.5°C of T_{pref} for *D. serrata* and the Paluma *D. birchii* population. The thermal optimum for Mt Lewis was found to occur 4.6°C above T_{pref} with overlapping confidence intervals (Fig.

4.5C). In addition, all temperature preference parameters (T_{pref} and T_{set}) were located within each B_{80} range, with the exception of T_{set} for the Mt Lewis *D. birchii* population, indicating that temperature preference falls within the thermal environments which maximize development speed for these species.

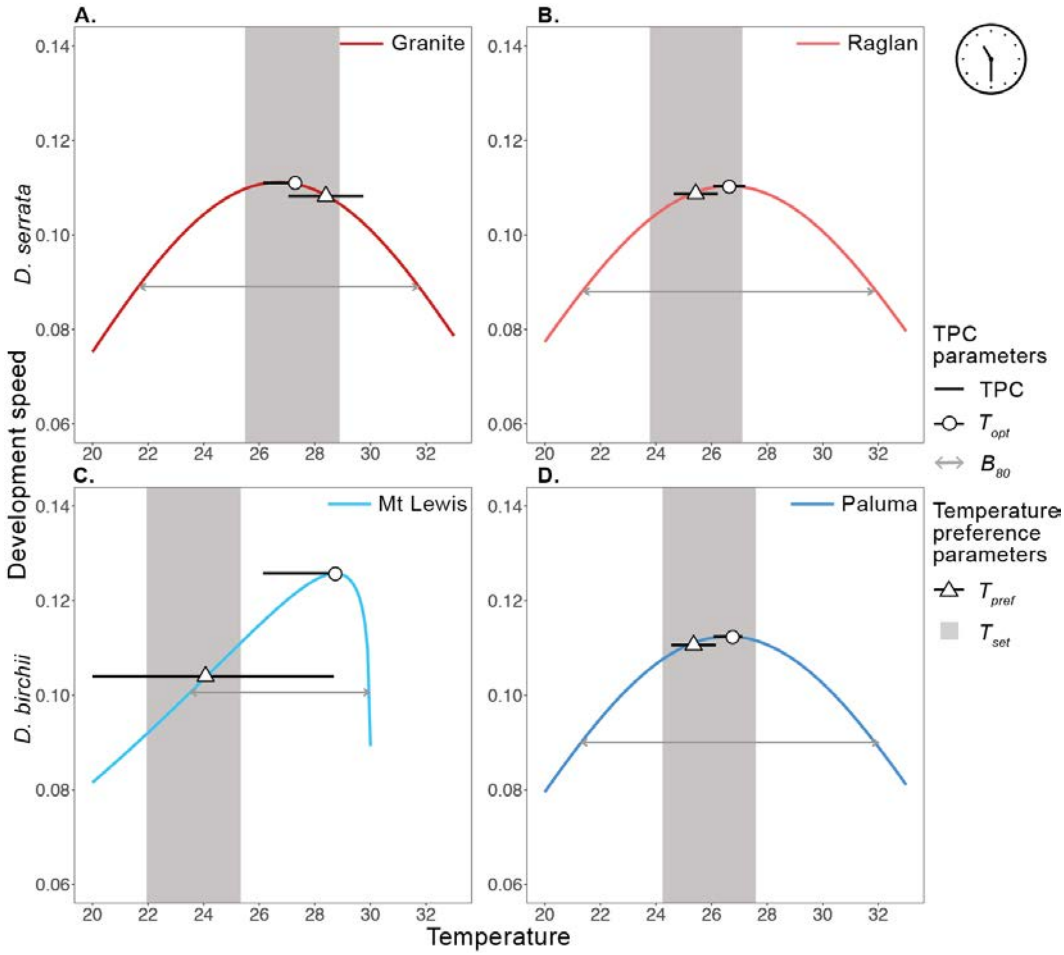

Figure 4.5: Comparison of development speed TPCs and temperature preference of a thermal generalist (red) and a thermal specialist (blue) *Drosophila* species.
Development speed TPCs for populations of *D. serrata* are shown in red (**A, B**) and *D. birchii* are shown in blue (**C, D**). TPC parameters shown include T_{opt} and B_{80}. Temperature preference parameters shown include where T_{pref} falls along the TPC and the 80% temperature preference range (T_{set}). 95% confidence intervals for T_{opt} and T_{pref} are shown as black error lines.

The thermal optimum for wing size does not correlate to oviposition temperature preference (r = -0.167, P = 0.833), and I did not find significant coadaptation between the two (R^2 = 0.03, $F_{1,2}$ = 0.06, P = 0.833). There was no overlap between the thermal optimum for wing size and temperature preference parameters for *D. serrata* (Fig. 4.6A, B). For wing size in *D.*

birchii, the thermal optimum fell just within the temperature preference confidence interval but outside of the T_{set} range for Mt Lewis, although the 95% confidence interval for T_{opt} overlapped with T_{set} and the 95% confidence interval for T_{pref} (Fig. 4.6C). For the Paluma population, the 95% confidence interval for T_{opt} only slightly overlapped with the lower confidence interval for T_{set} (by 0.4°C).

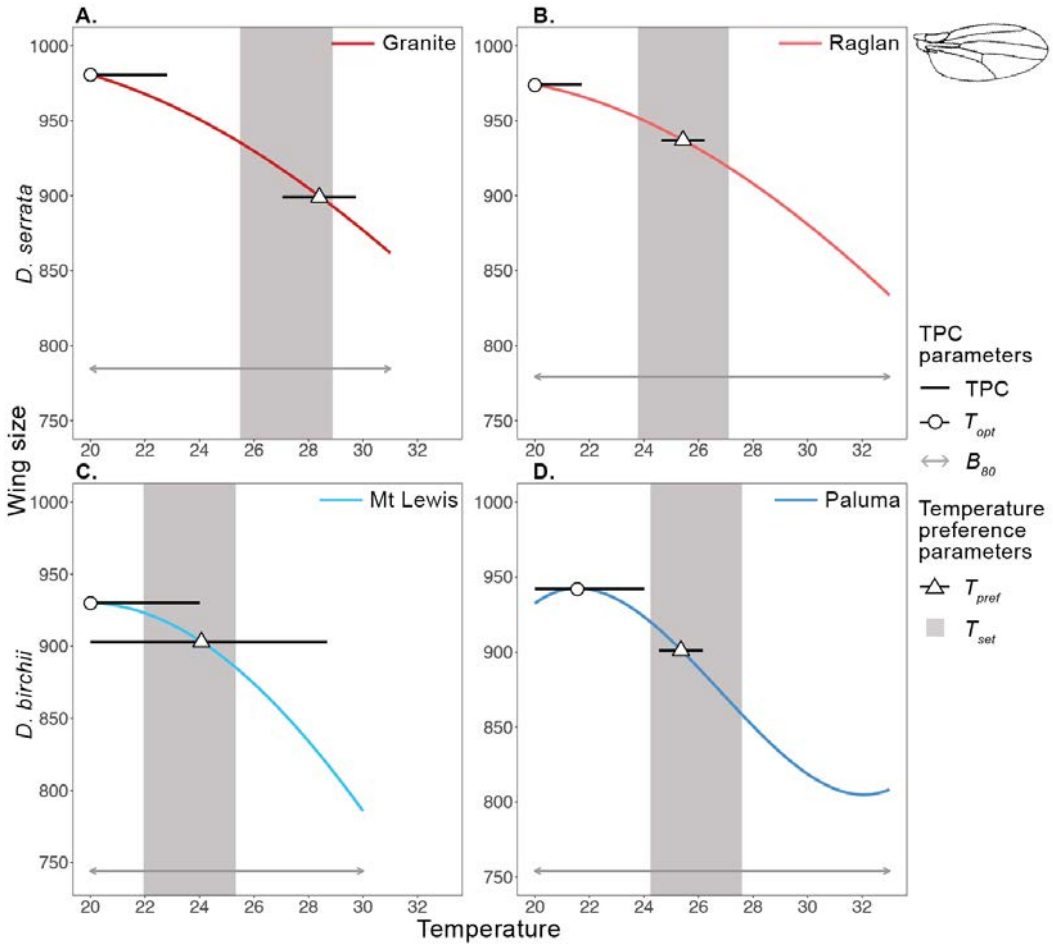

Figure 4.6: Comparison of wing size TPCs and temperature preference of a thermal generalist (red) and a thermal specialist (blue) *Drosophila* species.

Wing size TPCs for populations of *D. serrata* are shown in red (**A, B**) and *D. birchii* are shown in blue (**C, D**). TPC parameters shown include T_{opt} and B_{80}. Temperature preference parameters shown include where T_{pref} falls along the TPC and the 80% temperature preference range (T_{set}). 95% confidence intervals for T_{opt} and T_{pref} are shown as black error lines.

'Jack of all Trades' or 'Sub-optimally Risk-Averse'?

The 'jack of all trades' hypothesis predicts that thermal generalists will have a greater gap between T_{opt} and T_{pref} than in specialists. In testing this hypothesis, I did not find significant differences between species in any trait (one-sided t-test of Generalist T_{opt} - T_{pref} > Specialist T_{opt} - T_{pref}; *Productivity*: $t_{1.6} = 0.98$, $P = 0.775$; *Development speed*: $t_{1.8} = -1.49$, $P = 0.144$; *Wing size*: $t_{1.02} = -1.99$, $P = 0.146$). The 'sub-optimally risk-averse' hypothesis predicts that specialists will have a greater gap and be located at lower temperatures than in generalists. I also did not find significant differences between species when testing this hypothesis in any trait (one-sided t-test of Specialist T_{opt} - T_{pref} > Generalist T_{opt} - T_{pref}; *Productivity*: $t_{1.6} = 0.98$, $P = 0.224$; *Development speed*: $t_{1.8} = -1.49$, $P = 0.856$; *Wing size*: $t_{1.02} = -1.99$, $P = 0.854$).

In relation to the prediction of the 'sub-optimally risk-averse' hypothesis that oviposition temperature preference should be located at temperatures lower than the thermal optimum, I found T_{pref} was located at temperatures above the T_{opt} for productivity (*D. birchii*: 1.09°C ± 0.88 SD; *D. serrata*: 0.37°C ± 0.53 SD) and wing size (*D. birchii*: 3.94°C ± 0.19 SD; *D. serrata*: 6.91°C ± 2.09 SD) in both species, but below the T_{opt} for development speed in both species (*D. birchii*: -3.04°C ± 2.31 SD; *D. serrata*: -0.05°C ± 1.63 SD). However, these differences were non-significant when comparing the gap between oviposition temperature preference and thermal optimums between species ($t_{9.9} = -0.86$, $P = 0.409$).

4.5 Discussion

I investigated two competing hypotheses on whether temperature preference is more tightly coadapted to thermal performance in a thermal generalist or in a thermal specialist species of *Drosophila* by measuring oviposition temperature preference and comparing it to TPCs for productivity, development speed, and wing size. The first hypothesis predicts thermal generalists may exhibit a temperature preference that deviates more from peak performance temperature than in thermal specialists (Blouin-Demers et al., 2003). This is because generalists are thought to have a lower, but more stable, fitness at and around their thermal optimum and a wider niche breadth ('jack of all trades', but 'master of none'; Huey & Hertz, 1983; Fig. 4.1B). Consequently, generalists have more thermal environments to move in—and out of —to maintain close-to optimal fitness in the wild, and temperature preference would coadapt over a wider range of thermal environments than within specialists. The second hypothesis, which I term the 'sub-optimally risk averse' hypothesis, predicts that

thermal specialists should exhibit a temperature preference further below the thermal performance optima than in generalists because specialists should be more 'risk-averse' against overshooting their thermal optimum (Fig. 4.1C). I examined these hypotheses by first experimentally measuring whether a generalist *Drosophila* species exhibits a wider peak-oviposition temperature preference range (B_{80} for temperature preference) than a closely-related specialist *Drosophila* species (the 'jack of all trades' hypothesis); and second, by comparing the gap between oviposition temperature preference and thermal optimums in the generalist and specialist species ('jack of all trades' versus 'sub-optimally risk-averse' hypotheses).

I found no evidence to suggest that temperature preference range mimics the 'jack of all trades' hypothesis; where peak temperature preference may occur over a wider range of temperatures for a thermal generalist than for a thermal specialist. This is evidenced by the non-significant difference in temperature preference B_{80} between *D. serrata* (the generalist) and *D. birchii* (the specialist). Since generalists use a wider set of thermal environments with little associated cost to fitness, there is hypothesised to be less pressure to 'prefer' a specific temperature than within specialists. However, my study indicates that our generalist species exhibits a similar peak temperature preference range to our specialist species, suggesting that it is beneficial to confine temperature preference to a restricted range of thermal environments independent of being a thermal generalist or specialist. Our results corroborate those found by Blouin-Demers et al. (2003) who observed no difference in T_{set} range for a thermal generalist and a more precise thermoregulator.

Secondly, there was no significant difference in the deviation between temperature preference and thermal optimum between species for any trait—meaning I did not find evidence for either of the two hypotheses regarding how 'tightly' temperature preference may have coadapted to thermal performance in species with two different thermal strategies. I therefore am still unsure what is the main driver behind temperature preference range evolving with optimal performance range; whether: 1) the wider peak of optimal temperatures would cause an associated wider temperature preference range in generalists, or 2) the high cost associated with a temperature preference that is closer to T_{opt}, and subsequently closer to the upper thermal limit, causes a wider temperature preference range in specialists. I propose that the drivers underlying the two hypotheses are stabilizing and confine temperature preference to a similar range in both species, but more empirical research is needed to tease this apart.

When examining the TPCs individually, I confirmed that *D. serrata* is a thermal generalist in comparison to its sister species *D. birchii*. Yet, I did not find evidence of a trade-off between peak fitness and niche width for two fitness traits for *D. birchii*. Instead, I found that *D. serrata* exhibited both higher peak fitness and a wider B_{80} for productivity and wing size, while *D. birchii* exhibited a slightly higher maximum development rate than *D. serrata* (Fig. 4.3). I found evidence for a trade-off between development speed and body size when comparing across and within species, and this corroborates previous research showing that quicker development times result in smaller adult body sizes from consuming less resources during development (Chippindale et al., 1997).

When examining oviposition temperature preference individually, I found *D. birchii* preferred a mean temperature located, although insignificantly, 2.2°C cooler than *D. serrata*. This mirrors what was found for the thermal optimum for productivity and the insignificant result is most likely due to a lack a power associated with the analysis. Although previous research has found mixed results when comparing temperature preference to distributions (Schnebel & Grossfield, 1986; Krstevska & Hoffmann, 1994; Yamamoto, 1994; Matute et al., 2009), I expected this to be case based on the environments they live within in the wild. This is based on the assumption that these species are both well-adapted to their respective environments and preferences have coevolved with this adaptiveness. I believe this is a sound presumption for these species because previous research on *D. serrata* and *D. birchii* have found evidence of localized adaptation of tolerance and life history traits (Hallas et al., 2002; Hoffmann et al., 2003a; Sgrò & Blows, 2003; Griffiths et al., 2005; van Heerwaarden et al., 2009; Bridle et al., 2009) from latitudinal and altitudinal adaptation studies.

To my knowledge, my study provides the first example comparing thermal fitness optimums to oviposition preference site. From an evolutionary perspective, oviposition preference site should be equally as important as adult temperature preference in determining how well-adapted a species is to their natural environment. This is because natural selection favours females that can discriminate between lethal and favourable thermal environments because this directly affects survival of her progeny (Jaenike, 1978; Thompson, 1988; Mery & Kawecki, 2004; Gripenberg et al., 2010; Soto et al., 2011). Female *Drosophila* have been shown to detect and choose oviposition site by ‘probing’ the substrate before laying (Yang et al., 2008), and have been shown to oviposit based on the current temperature of the substrate (Schnebel & Grossfield, 1986; Fogleman, 1979). When examining sister-species, as I am here, a divergence in oviposition preference site could potentially reflect either a basis for, or a consequence of, the speciation process, in addition to selection imposed by their different

habitats. Of course, a limit to our study was that I was not able to control for detrimental developmental effects of temperature on eggs once they were laid. However, I believe the method used here is most representative of what occurs in the wild (females choose oviposition site, individuals develop within that fixed thermal environment, and the combination of these effects is what leads to overall reproductive success). In addition, it is currently unknown how congruent adult female temperature preference is with oviposition temperature preference (Dillon et al., 2009), with some evidence suggesting oviposition temperature preference could be a reflection of adult temperature preference (or vice versa; Schnebel & Grossfield, 1986). Additional research is needed on both to determine their influence on one other.

A second main goal of my study was to test the 'thermal coadaptation hypothesis' between important fitness traits and oviposition temperature preference. Most importantly, I found temperature preference and productivity to be an example of ''tight' coadaptation (slope of regression = 1.19), most likely because it is a direct measure of reproductive success. This provides evidence that temperature preference has coadapted directly alongside ultimate fitness traits. Although the thermal coadaptation hypothesis was built upon the assumption that temperature preference should adapt tightly to thermal performance of fitness, evidence for coadaptation between ultimate fitness traits and temperature preference is scarce (Halliday & Blouin-Demers, 2015; Halliday & Blouin-Demers, 2017). This is because direct fitness is notoriously hard to measure. This result has important implications for understanding the effect of behaviour on the adaptive potential of fitness under climate change. I show that temperature preference is most tightly linked to thermal environments where ultimate fitness traits are optimal. This is important because many species exhibit localized adaptation, indicating that species that are able to thermoregulate will use behaviour to move to thermal environments most similar to their habitual, unchanged environment and this may hinder adaptation to novel conditions in important fitness traits.

In contrast, I found no evidence for coadaptation between the thermal optimum for development speed and wing size, indicating that selective pressure to maintain optimal development speed and a large wing size may be less than what occurred for productivity. Although development speed is considered an ultimate fitness trait, these species are not known to be 'rapid developers' in comparison to similar sized *Drosophila* (e.g., Jenkins and Hoffmann 1999). Hence, selection on development speed may weak because it may not be an important contributor to fitness in these species. In addition, temperature preference was measured from only three replicates for Mt Lewis (due to an equipment malfunction and

subsequent results being unreliable), which introduced uncertainty surrounding the temperature preference estimates for Mt Lewis. As such, temperature preference had to be estimated from unweighted curves as weighting the data-points along the thermal gradient did not correspond to the fit of the data (Appendix C Figure 4).

Future directions

The 'thermal coadaptation hypothesis' states that the thermal optimum for fitness should correlate to the temperature preference of a species to maximize fitness at temperatures experienced in their natural thermal environment (Huey & Bennett, 1987; Huey & Kingsolver, 1989; Angilletta, 2009). Understanding the mechanisms behind coadaptation between temperature preference and thermal optimums would allow us to better predict how species will respond to changing temperatures associated with global warming. This is important because a large component of current climate change research is understanding how species will react—will a species change distributions, adapt, or concede to the changing conditions? Researchers investigate this problem by mapping the thermal niche of a species and comparing it to climate change predictions. Currently, only 36% of publications use TPCs to describe the thermal niche, whereas 10% use solely thermal preference and the majority (47%) use distributional data to characterise a species thermal niche (Gvoždík, 2018).

Here, I show that oviposition temperature preference is coevolved with the thermal optimum for an important fitness trait (productivity) in these populations, confirming that both oviposition temperature preference and thermal physiological performance contribute to a species thermal niche. This means that both behaviour and thermal physiological traits need to be included into model predictions if researchers want to accurately describe how an ectotherm will respond to changing temperatures. However, few are currently doing so. Not only does this have implications for developing species distribution models in a changing climate, but it is thought that thermal preferences may actually buffer adaptation to rising temperatures. This may occur because species can use behaviour to move to their preferred thermal environments, slowing down the rate of adaptation to the raw environmental conditions (known as the 'Bogert effect'; Bogert 1949; Buckley et al., 2015)

If we know more about a species temperature preferences, and how it relates to fitness, we can better predict how behaviour can change the direction of adaptation. One way of understanding this would be to investigate whether temperature preference is heritable and

under what conditions. Temperature preference has been shown to be heritable in some species of *Drosophila* (see Dillon et al., 2009 for review). However, most recently, Castañeda et al. (2019) found thermal preference had a low heritability in *D. subobscura* (h^2 = 0.07). Yet, other studies found evidence for local adaptation of temperature preference to latitudinal variation for this species (Huey & Pascual, 2009; Castañeda et al., 2015), indicating temperature preference may partially respond to local conditions. In order to decipher whether temperature preference is coadapted with thermal optimums as a result of behaviour or genetic covariation and linkages, heritability and genetic covariances for both traits would need to be estimated for our species. If temperature preference is genetically correlated to fitness, a shift in one would cause a shift in the other, potentially benefiting species in changing climates (Huey & Bennett, 1987; Huey & Kingsolver, 1989).

A major application of the data identified here would be if temperature preference was found to be coadapted to fitness traits within and across genera. Widespread taxonomic correlation could be used to identify optimal thermal habitat of vulnerable or endangered species. This is important because performance and/or tolerance tests are not usually permitted on listed threatened species due to the stress, and potential mortality, they induce. Here, I found that the level of coadaptation was maintained across three fitness traits in two sister-species, giving support to the thermal coadaptation hypothesis. In one of the first empirical studies on the 'thermal coadaptation hypothesis', Huey and Bennet (1987) proposed three different situations that may result from coadaptation of temperature preference and performance: 1) 'perfect coadaptation', where the shift in thermal optimum and preference is a near 1:1 match between the ancestral species and the adapted species; 2) 'partial co-adaptation', where thermal optimum and temperature preference adapt in the same direction but one evolves less rapidly than the other, and; 3) 'antagonistic co-adaptation', where the thermal optimum for performance and temperature preference adapt in opposite directions of each other. By incorporating phylogeny and a greater number of species from the *montium* subgroup of *Drosophila*, I could decipher whether 'perfect', 'partial' or 'antagonistic' coadaptation has occurred. Importantly, these results indicate that the thermal optimum of traits more closely related to reproductive output (productivity) will coevolve with preferences at a more similar rate than those not (development speed and wing size). For researchers looking to identify optimal thermal habitat of vulnerable species, they can first see if temperature preference and tolerance data is available for ultimate fitness traits in a

related-common species and use this correlation to predict the relationship between their species' temperature preferences and optimal habitat.

4.6 Highlights

- I investigated the thermal coadaptation hypothesis in a generalist and a specialist species of *Drosophila* to see how thermoregulatory behaviour is related to the optimum thermal-performance temperature of key fitness traits.
- I found oviposition temperature preference is tightly coadapted to the optimum thermal temperature for productivity; an ultimate fitness trait.
- I found that peak temperature preference range did not differ between a thermal generalist and a thermal specialist.
- The temperature an organism prefers may offer a good proxy to the optimal temperature for ultimate fitness traits.

Chapter 5: Surviving a heatwave does not future-proof populations for the next heatwave

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Contributions: JC, CH, and MH conceived the experiment. JC conducted the experiment and collected data. JC analysed data, with important contributions from MH. EO provided support for the heritability analysis. JC, MH, and CH wrote the manuscript.

5.1 Abstract

Extreme heat events are increasing in frequency, intensity, and duration as a result of climate change. These ‘heatwaves’ may have greater impacts on ecological communities than gradual warming. Heatwaves that cause mortality are thought to act as hard selection events for individuals with increased heat tolerance and theory suggests this should result in subsequent generations better adapted to withstanding future heatwaves. I show this is not the case. I first measured a heritable thermal tolerance trait (knockdown time) in isofemale lines of two wild *Drosophila* populations, and selected for high tolerances to mimic a population immediately after a moderate and after a severe heatwave. Between ‘heatwave seasons’ (i.e., one year) there was no further selection. Then I subjected heatwave-selected populations to a second artificial heatwave and measured fitness traits (productivity, development speed, and wing size) along a thermal resource gradient to create thermal performance curves. I found populations affected by an initial heatwave (both moderate and severe) demonstrated no increased thermal tolerance (knockdown time) to a subsequent heatwave and also exhibited reduced fitness. I found that both a moderate and severe heatwave significantly decreased overall productivity and wing size, but did not affect development speed. Specifically, when looking at thermal performance curves, I found the peak fitness value for productivity significantly decreased within both heatwave survivor populations, while all other parameters for productivity, development speed, and wing size did not significantly change. These results suggest that heatwaves may cause maladaptation and that we cannot presume heatwave survivors will be better adapted to future heatwaves; an alarming conclusion given climate change predictions.

5.2 Introduction

Heatwaves are extreme temperature events characterised by prolonged periods of excessive heat (Perkins & Alexander, 2013; Perkins-Kirkpatrick & Lewis, 2020). In many species, these can cause sudden mass-mortality where thousands of individuals succumb to the extreme conditions in a very short timeframe (Welbergen et al., 2008; Garrabou et al., 2009; McKechnie & Wolf, 2010). For example, 33% of the Australian population of Spectacled Flying Foxes died in less than two days following a heatwave in Queensland in November 2018 (Fig. 5.1A). Over the past century, heatwaves have increased in frequency, intensity, and duration (Perkins-Kirkpatrick & Lewis, 2020; Fig. 5.1B), and are expected to worsen—current 20-year events are predicted to occur every two years (Collins et al., 2013). Consequently, heatwaves may pose an even greater risk to ecological communities (e.g., Allison, 2004; Hance et al., 2006; Mouthon & Daufresne, 2006; Jöhnk et al., 2008; Garrabou et al., 2009; McKechnie & Wolf, 2010; Sorte et al., 2010; Smale & Wernberg, 2013; Wernberg et al., 2013; Ma et al., 2015; Carreira et al., 2016; Carreira et al., 2017; Straub et al., 2019; Zhu et al., 2021) than the gradual rise of temperatures expected with global warming (Hance et al., 2006; Vasseur et al., 2014).

Yet, until recently, the majority of global change research has focused on predicting long-term effects of gradual climate change while overlooking the more sudden impacts of extreme heat events (Chapman et al., 2014; Vasseur et al., 2014; Carreira et al., 2016). While gradual climate change can seriously affect the abiotic and biotic environment, individuals may have the chance to adapt, disperse, or generate beneficial phenotypic plasticity (Hoffmann & Sgrò, 2011). On the other hand, heatwaves will test the immediate response of an individual's tolerance and/or its ability to rapidly acclimate or behaviourally regulate (Reusch et al., 2005; Carreira et al., 2016; Stillman, 2019). It is generally assumed that heatwave survivors will produce populations that are heat adapted in some sense, and hence will be relatively better adapted to future heatwaves (Clusella-Trullas et al., 2011; Overgaard et al., 2014; Stillman, 2019). This is theoretically intuitive given that the survivors did not succumb to the extreme conditions and the traits that facilitated the response are likely to be heritable; thus adaptation can occur (Endler, 1986; Falconer & Mackay, 1996; Gilchrist & Huey, 1999). Recently, the long-term outcome of an increased thermal tolerance as a result of a heatwave has been termed the 'silver-lining' effect of heatwaves (Coleman & Wernberg, 2020); because presumably individuals with relatively higher heat-tolerance will fare better under global change scenarios.

Empirical research has found that within-generation acclimation affects an individual's response to an extreme heat event within an individual's lifetime; and can either reduce performance and survival in a heatwave (Siegle et al., 2018; Aspinwall et al., 2019) or produce a short-term 'silver-lining' by inducing plasticity and increased thermal tolerance (Bauweraerts et al., 2013; Drake et al., 2018). These studies are important for understanding the short-term (within an individual's lifetime) implications of heatwaves, but do not assess how heatwaves affect subsequent generations, and whether there are long-term evolutionary 'silver-linings'. Many long-term studies have examined thermal tolerance evolution from acute heat events by performing selection on thermal tolerance traits *generation-after-generation* to assess genetic and fitness trade-offs (e.g., McColl et al., 1996; Gilchrist & Huey, 1999; and reviewed in Hoffmann et al., 2003b), which is fundamental to understanding adaptation in thermal tolerance traits. However, these results are not directly transferable to heatwaves because heatwaves often occur during 'heatwave seasons' (Perkins-Kirkpatrick & Lewis, 2020)—meaning species with short generation times undergo multiple generations of *benign conditions* following the first heatwave before being affected by the next heatwave event.

What is currently lacking is an understanding of the long-term effects heatwaves may have (Bailey & Pol, 2016), and how being exposed to one extreme heat event can adversely affect adaptation to subsequent heatwaves when these occur many generations later, and long-term fitness (but see Sentis et al., 2016, Sales et al., 2018, Leicht and Seppälä, 2019, Miler et al., 2020, and Waltzer et al., 2020 for important insights into short-term, transgenerational effects of heatwaves on fitness of both parents and their offspring; and see Coleman et al., 2020 and Gurgel et al., 2020 for recent insights into long-term fitness effects of heatwaves within the marine environment). Understanding this is imperative considering climate models are predicting current heatwave trends to worsen (Collins et al., 2013; Perkins-Kirkpatrick & Lewis, 2020). To my knowledge, what has not been conducted is an experiment investigating the impact of a heatwave on fitness and the performance of a population in a subsequent heatwave after many generations of benign conditions (i.e., no selection on heat tolerance; but see Zhu et al. 2021 for a recent experiment testing the effects of a simulated heatwave on thermal tolerance after three generations of benign conditions).

In this study, I tested whether a population affected by a heatwave exhibits an increased heat tolerance many generations after the extreme heat event. If heat tolerance is heritable, when unable to behaviourally regulate, individuals that survive a heatwave will pass on a higher heat-tolerance to their offspring than those that did not survive (Endler,

1986; Falconer & Mackay, 1996; Gilchrist & Huey, 1999). Based on this, we artificially recreated this situation by making heatwave treatment populations of *Drosophila birchii* after a moderate heatwave and after a severe heatwave, which were founded from individuals with known and varying degrees of heat-tolerance that we show to be highly heritable. Approximately one year after the original 'heatwave' (i.e., heatwave 1), I evaluated the populations' performance in a second, comparable heatwave (i.e., heatwave 2) to test the prediction that heatwave selection, caused by heat-induced mortality, will increase population-level tolerance during a subsequent heatwave (Fig. 5.1C).

In addition, I examined the broader impact of heatwaves by looking at the effect of a wide range of temperatures on important fitness traits in heatwave impacted populations. I created thermal performance curves (TPCs) for productivity (total offspring) and reaction norms for development speed (how quickly an individual develops from egg to adult) and adult body size. TPCs quantify an individual's performance and reaction norms assess developmental traits along a temperature gradient; both are considered fundamental to theoretically and conceptually understanding thermal adaptation (Huey & Kingsolver, 1989; Gilchrist, 1995; Angilletta, 2009). They are characterised by the following biologically-relevant parameters. Thermal optimum (T_{opt}): the temperature where peak performance (P_{max}) occurs. Thermal breadth (B_{80}): the range of temperatures where performance is above 80% peak performance (hence a measure of specialisation). Critical thermal limits (CT_{min} and CT_{max}): the points where fitness reaches zero (hence indicating thermal limits (Huey & Stevenson, 1979; Fig. 5.1D).

The creation of TPCs allowed me to answer two questions: first, does the upper thermal limit (i.e., CT_{max}) increase after a heatwave and with the severity of a heatwave; and second, what is the overall, long-term effect heatwaves have on population-level fitness. Following theory, I predicted that heatwave survivor populations will exhibit an upward shift in CT_{max} due to an increase in their mean heat tolerance and overall upward shift of TPC (Huey & Stevenson, 1979; Buckley & Huey, 2016; Fig. 5.1D). I also predicted that, after initial recovery and in the long-term, there should not be an overall change in peak fitness (P_{max}), but that T_{opt} and B_{80} should increase along the temperature gradient due to an overall shift in TPC (Fig. 5.1D).

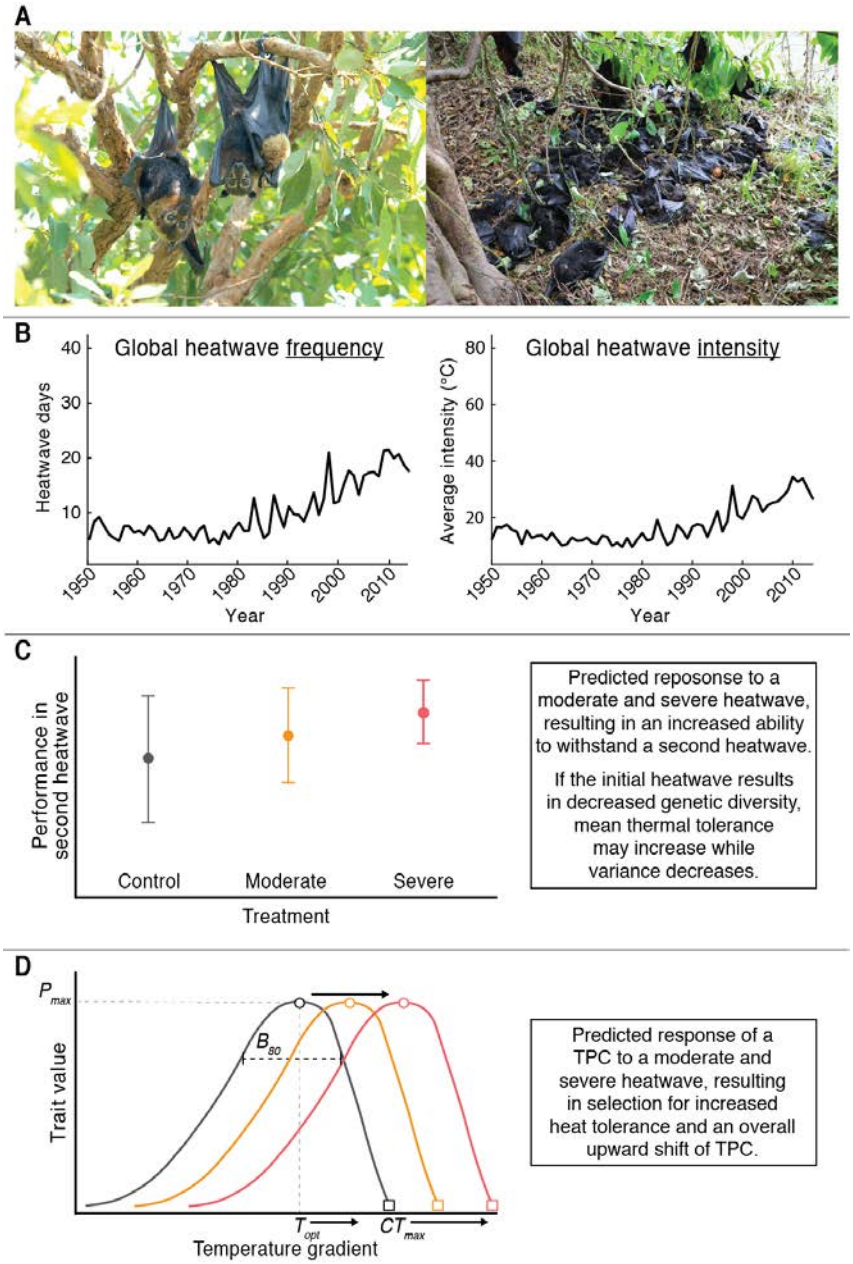

Figure 5.1: **Heatwave mortality, increasing severity, and predicted effects on heat tolerance.** **A**. Spectacled flying foxes (*Pteropus conspicillatus*) in Australia before and after an extreme heatwave that killed one-third (23,000 individuals) of the Australian population in only two days in 2018 (Hildebrandt, 2019). Photographs were taken and provided by J. Welbergen. **B.** Global trends in heatwave frequency (defined as the sum of all heatwave days) and heatwave intensity (defined as the average intensity across all heatwave days) for the past seventy years (Perkins-Kirkpatrick & Lewis, 2020). Figures modified from Perkins-Kirkpatrick & Lewis, (2020). **C.** Predicted response of populations affected by no heatwave (control), a moderate heatwave, and a severe heatwave to a subsequent heatwave approximately one year later. The control indicates no mortality in the initial event, a moderate heatwave indicates mortality of all but the upper 50% of heat tolerant individuals in the initial event, and a severe heatwave indicates mortality of all but the top 10% of heat tolerant individuals in the first event. Performance is measured as the amount of time individuals are able to survive in a subsequent heatwave. **D.** Predicted response to a heatwave showing a population unaffected by heatwaves (black curve) and the adapted response of a thermal performance curve after a moderate heatwave (orange curve) and after a severe heatwave (red curve). Figure adapted from Huey & Kingsolver (1993).

Heatwave treatment populations were established from two wild populations of *Drosophila birchii* (Fig. 5.2A). Flies were collected from two mountaintops located in northeast Queensland, Australia (Fig. 5.2B) to provide geographic replication. From these, I assessed the performance of twelve isofemale lines (i.e., a genetic line created by breeding the offspring of one female together) from each wild population during an acute heat-stress event to mimic a heatwave (i.e., heatwave 1). I used a static heat knockdown assay to assess the length of time an individual is able to tolerate extreme heat before becoming immobile, which is known as knockdown time (KD_T). Static heat knockdown assays have consistently predicted adaptive potential of upper thermal limits in *Drosophila* (van Heerwaarden & Sgrò, 2013; Blackburn et al., 2014; van Heerwaarden et al., 2015). They are relevant when directly assessing thermal tolerances because they test how individuals respond to acute conditions without confounding effects on physiology that occur during ramping assays (i.e., starvation and desiccation effects; Sgrò et al., 2010; Santos et al. 2011; Terblanche et al., 2011). The heatwave treatment populations were: 1) control populations bred from offspring of the isofemale lines with the five highest and five lowest mean KD_T where no heat-induced mortality occurred (Fig. 5.2C); 2) populations bred from offspring of the isofemale lines with the five highest mean KD_T, comprised of individuals who could survive 23% longer than the control (top 50% of heat-tolerant individuals; Fig. 5.2D), and 3) populations bred from the isofemale line with the single highest-mean KD_T where individuals could withstand extreme heat for 35% longer than the control (top 10% heat-tolerant individuals; Fig 5.2E). These represented a control treatment, a moderate heatwave treatment, and a severe heatwave treatment, respectively.

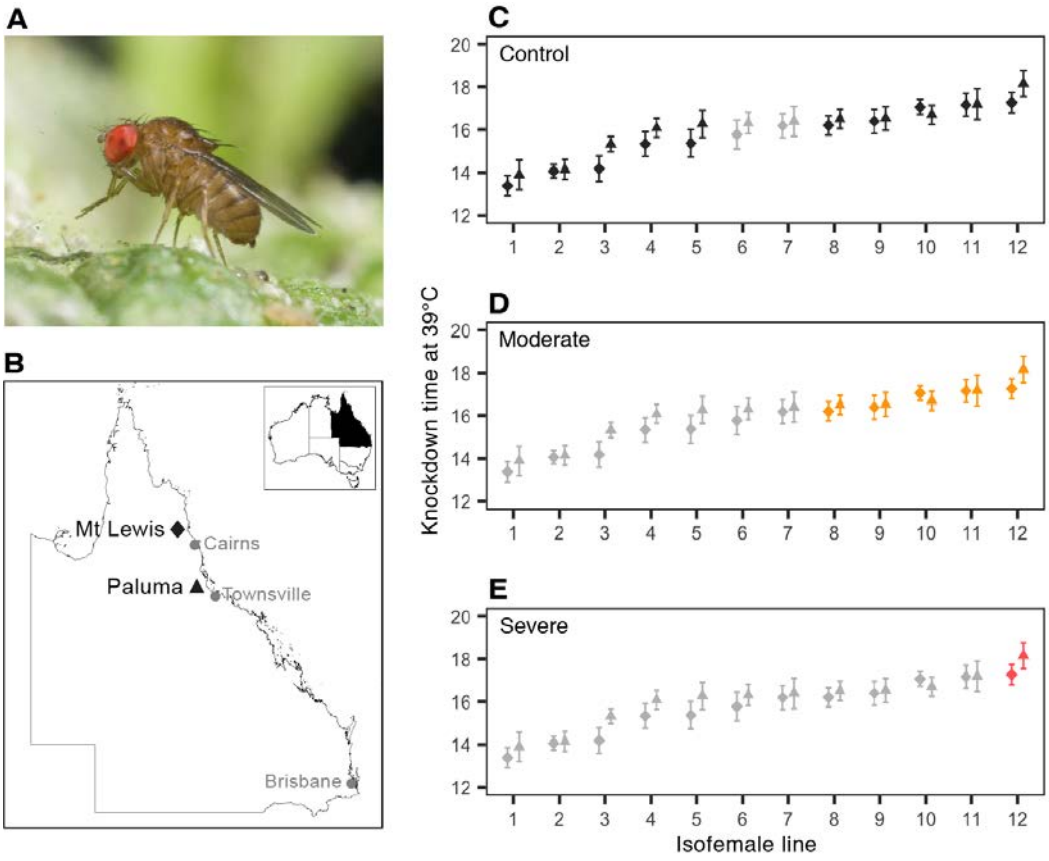

Figure 5.2: **Varying levels of heritable heat-tolerance in a native Australian species of *Drosophila*.**

A. *Drosophila birchii*. Photograph credit to A. Weeks. **B.** Map of Queensland, Australia showing collection sites for experimental flies from Mt Lewis and Paluma National Parks; and (**C–E**) static heat knockdown times of isofemale lines for each wild population *(N* = 20 per line). **C.** The isofemale lines with the five highest and five lowest knockdown times were bred together to create a 'control' population not affected by a heatwave; **D.** the isofemale lines with the five highest knockdown times were bred together to create a 'moderate' heatwave treatment, and, **E.** the offspring from the isofemale line with the single highest knockdown time were bred together to create a 'severe' heatwave treatment.

I confirmed that heat KD_T can respond to selection by calculating the heritability for each wild population from a variance component analysis. This allowed us to analyse the variance within and between isofemale lines to estimate the relative proportion of genetic variance of KD_T within each study population—a method that has been shown to calculate similar heritabilities to those estimated during a full sib analysis (Hoffmann & Parsons, 1988b). I confirmed a high heritability for each population (Mt Lewis; H^2 = 0.60, 95% CI [0.53, 0.73] and Paluma; H^2 = 0.49, 95% CI [0.14, 0.73]: Appendix D Table 1). This supports previous

research that found significant evolutionary potential for increased heat tolerance from static heat knockdown assays (van Heerwaarden & Sgrò, 2013), and that showed adaptation of the upper thermal limit is more likely to occur when selective pressure arises from an acute-stress thermal event rather than gradual warming (Blackburn et al., 2014).

5.3 Results

Thermal tolerance assessed as heat knockdown time

To test performance during a subsequent heatwave ('heatwave 2'), I maintained experimental populations in the laboratory at large sizes ($N > 1000$) for approximately one year (25 generations) and no further selection was placed upon them. I chose to separate heatwaves by one year to represent the time between heatwaves that occur in successive summers (i.e., heatwave seasons (Perkins-Kirkpatrick & Lewis, 2020). Geographical regions differ in the typical number of heatwaves experienced, from less than one heatwave per year to many more (Perkins & Alexander, 2013), and I decided to test the legacy of a heatwave over the longest typical time-lag (i.e., annual). Although I recognize that laboratory adaptation could have occurred over this period, previous studies have found little difference in both stress-tolerance and life-history traits of *Drosophila* between laboratory-maintained and recently-collected flies (Maclean et al., 2018). Specifically, CT_{max} has been found to have a high correlation, and no statistical difference, between field-caught and laboratory-kept populations in *D. birchii* (Maclean et al., 2018).

After one year, each heatwave population was subjected to a second heat knockdown assay ('heatwave 2'). I found heat knockdown times in 'heatwave 2' did not significantly differ between treatments. Model estimates indicate populations affected by a moderate heatwave had an average decrease in KD_T of 0.141 mins (± 1.014 SD; 95% CI: -0.647, 0.448) but this was not significantly different from the control population (19.91 ± 1.03 SD; CI: 18.73, 21.12; $P = 0.621$). Populations affected by a severe heatwave had an average increase in KD_T of 0.404 mins (± 1.015 SD; 95% CI: -0.15, 1.05) but this was also not significantly different from the control population ($P = 0.163$; Fig. 5.3 and Appendix D Table 2).

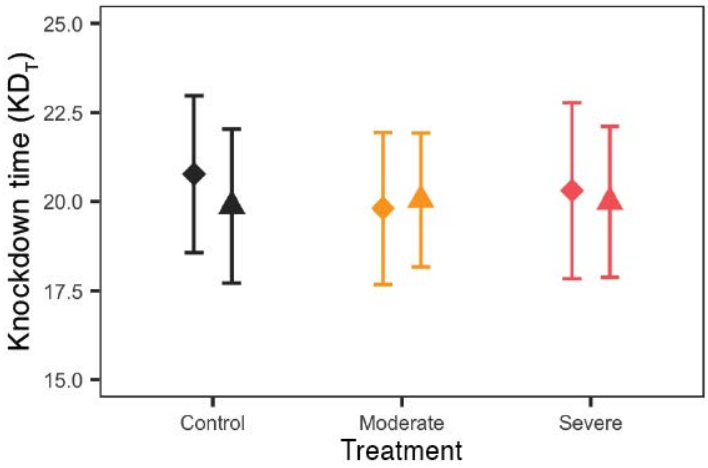

Figure 5.3: **Heatwave impacted populations are not better adapted for the next heatwave.** Performance of heatwave populations in a second heatwave. Performance was measured as the length of time individuals could withstand extreme heat (KD_T). Population replicates are denoted by a diamond for Mt Lewis and a triangle for Paluma. N = 200 per treatment.

Thermal tolerance and fitness measured from thermal performance curves

To assess how heatwaves affect the long-term fitness of populations, I measured productivity (total offspring that emerged from each vial), development speed (inverse of development time), and wing size (centroid size) one year after an initial heatwave (i.e., heatwave 1), and across a thermal resource gradient ranging from 20°C–36°C in 1°C intervals. Temperatures incorporated the higher end of the temperature range experienced throughout the geographical distribution of *D. birchii* and up to extremely stressful temperatures that may be experienced with worst-case climate change scenarios (Overgaard et al., 2014). I compared the overall effects of treatment by modelling trait value as a function of treatment type, temperature, population, and replicate. TPC parameters were estimated and compared across treatments (N = 8 per treatment) using non-linear least square (nls) models (Appendix D Tables 3–5; Appendix D Figures 1–3) fit to pre-defined functions (Appendix D Table 6).

Heatwave severity had a significant effect on overall productivity (X^2_2 = 39.3, P < 0.001), with the moderate heatwave having no significant effect (0.856 ± 0.103 SD rate-ratio on log scale; CI: 0.676, 1.09; P = 0.189) and the severe heatwave inducing a strong effect (0.419 ± 0.062 SD rate-ratio on log scale; CI: 0.313, 0.56; P < 0.001; Appendix D Table 7). TPC parameters estimated from the best-fit nls model (Fig. 5.4A) indicated a moderate heatwave significantly decreased productivity P_{max} by an average of 15.9 total offspring (±

3.57 SD; $F_{2,3} = 62.51$, $P = 0.022$) and a severe heatwave significantly decreased P_{max} by 31.1 offspring (± 3.19 SD; $F_{2,3} = 62.51$, $P = 0.003$; Fig 5.4G). Productivity T_{opt}, CT_{max}, and B_{80} were not significantly affected by either the moderate or severe heatwaves (Fig 5.4D, G and see Appendix D Table 8 for test of main effects and post-hoc comparisons).

Rapid development speed is considered an important fitness trait in *Drosophila* because the environment where they develop is considered transient (i.e., rotting fruit) and the slower they develop, the more competition they will experience from other larvae (Nunney, 1996; Chippindale et al., 1997). Here, I found development speed was not influenced by either a moderate or severe heatwave ($X^2_2 = 0.873$, $P = 0.65$; Appendix D Table 9). The development speed reaction norms (Fig. 5.4B) indicated P_{max} (i.e., the quickest development rate), T_{opt} (i.e., the temperature where the quickest development time occurred), and B_{80} (i.e., the range of temperatures where the quickest 20% of development occurred) did not change from the control to the heatwave treatments (Fig. 5.4E, H and see Appendix D Table 8 for test of main effects and post-hoc comparisons).

A trade-off has been shown to exist between development time and body size, where a quicker development time often means lower overall resource intake and therefore smaller adult individuals (Chippindale et al., 1997). Here, I can clearly see a pattern between development time and wing size (a proxy for body size) that indicates a trade-off may exist (Fig. 5.4B, C; for opposing evidence see Sgrò & Hoffmann, 1998b). Overall, wing size was significantly affected by heatwave treatment ($X^2_2 = 49.899$, $P < 0.0001$; Table Appendix D 10). However, similar to development speed, P_{max} (i.e., largest wing size), T_{opt} (i.e., the temperature where wing size was the largest), and B_{80} (i.e., the range of temperatures where the largest 20% of wings occurred) were not significantly affected by heatwave treatment (Fig. 5.4F, I and see Appendix D Table 8 for test of main effects and post-hoc comparisons).

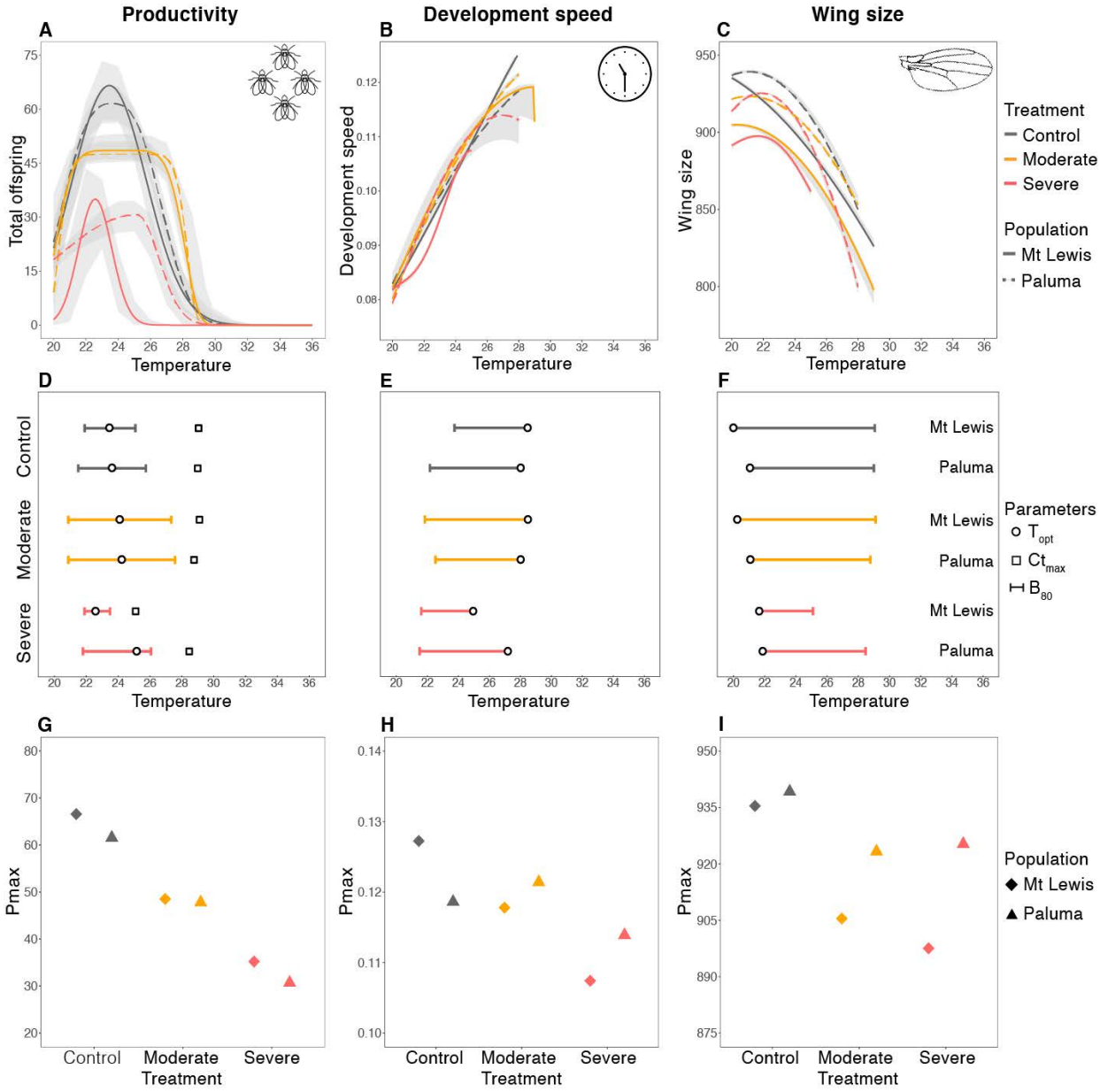

Figure 5.4: Heatwave results in long-term maladaptation.

TPCs and reaction norms for fitness traits measured one year after populations were affected by heatwaves. **A–C.** Estimated curves for productivity, development speed, and wing size for each heatwave treatment and wild population with 95% confidence intervals for each curve shown in grey. **D–F**. TPC parameters for heatwave treatments and wild populations across the thermal gradient. **G–I.** Peak performance (P_{max}) for each fitness trait. P_{max} represents the highest number of offspring that emerged (productivity), the quickest development time, and the largest wing size.

5.4 Discussion

Our results suggest that a heatwave does not necessarily act as a hard selection event on thermal tolerance. This is notable, as it is currently theorized that extreme events that cause mortality-based selection will enhance future tolerance (Grant et al., 2017); termed a 'silver-lining' effect where populations are better suited to withstanding future rapid climate change (Coleman & Wernberg, 2020). Here, I tested the 'silver-lining' hypothesis by selecting for heat-tolerant phenotypes and examining thermal tolerance generations after selection occurred; but I found no adaptation to heat stress. In fact, I found that populations impacted by heatwaves were adversely affected by the heatwave in a classic example of maladaptation (Brady et al., 2019), as evidence by reduced fitness (in productivity and wing size).

Additionally, the maximum performance of productivity (the trait most directly related to ultimate fitness) decreased relative to severity of the heatwave (Fig. 5.4G). This indicates that the long-term damage incurred on population-level fitness may be directly proportional to the intensity of a heatwave. How acute thermal-intensity proportionally affects physiological processes has been modelled in previous papers (e.g., Santos et al., 2012), but there has been no empirical investigation into the relative effects of thermal intensity on long-term fitness to date (but see Carreira et al., 2016 for investigation into the effects of heatwave-duration on within-generational fitness). This has important implications for future climate change research, suggesting that research should now focus on the intensity of the heatwaves in addition to including gradual warming when investigating possible consequences of climate change.

Although our study did not find evidence for adaptation of thermal tolerances following a heatwave, a recent field study confirmed that a 'silver-lining' is possible when they found that an extreme winter event caused adaptation for cold tolerance in lizards (Campbell-Staton et al., 2017). Additionally, a study found an increase in heat-tolerant alleles following a marine heat event in seagrasses, but in only a portion of impacted geographical-areas (Coleman et al., 2020). Overall, long-term studies on the impacts of extreme heat events have been scarce compared to short-term, descriptive studies that investigate individual responses (for review, see Bailey & Pol, 2016); and more evidence is needed to understand the underlying mechanics that may, or may not, produce a 'silver-lining' after an extreme heat event.

Here, I suggest that maladaptation occurred and adaptation was constrained due to an overall decrease in genetic diversity within each population caused by the population

bottleneck at the point of the heatwave, and a subsequent increase in inbreeding depression. However, we did not measure genetic diversity before and after the heatwaves to directly assess this. Loss of genetic diversity and an increase in inbreeding has been shown following a marine heatwave in seagrasses (e.g., Coleman et al., 2020; Gurgel et al., 2020) and high genetic diversity has been found to be beneficial for recovery after a marine heatwave in seagrasses (e.g., Reusch et al., 2005; Wernberg et al., 2018). Another explanation for the results found here is that the individuals in the heatwave-impacted populations retained the alleles responsible for the highly-heritable heat stress trait but did not express increased heat tolerance due to adverse fitness effects (e.g., Hance et al., 2006; Mouthon & Daufresne, 2006; Jöhnk et al., 2008; Garrabou et al., 2009; McKechnie & Wolf, 2010; Sorte et al., 2010; Carreira et al., 2016; Carreira et al., 2017; Straub et al., 2019). Although logistically-challenging to do in the wild, empirical studies should be conducted that focus on the underlying genetics of populations before and after an extreme event to decipher if maladaptation and limited adaptive potential in thermal tolerance is linked.

In addition to the genetic-architecture of a population, the possibility for recovery and survival in the wild after an extreme event will also depend on population size (Bell, 2012) and recent stresses (Gonzalez & Bell, 2012). I maintained the populations at large sizes and under benign conditions after the initial heatwave, so these were not detrimental factors. However, this will most certainly not be the case for many wild populations. For example, I examined heatwaves that occurred one-year apart, but current trends indicate the number of heatwaves per season is increasing (Perkins-Kirkpatrick & Lewis, 2020)—meaning populations may be effected by multiple heatwaves in one year which may compound adverse fitness effects. If further research finds that decreased genetic diversity following a heatwave is affecting population fitness and constraining evolution of stress-tolerant traits, then maintaining connectivity and creating artificial gene flow between populations following a heatwave may offer a path forward for managing adverse effects. Here, I offer novel empirical research into the outcomes of selection from an extreme heat event. However, large gaps remain in understanding the long-term evolutionary consequences of such events in nature.

5.5 Methods

Study species

Drosophila birchii is a specialist species of tropical Drosophila endemic to north-eastern Australia and Papua New Guinea (Schiffer & Mcevey, 2006). In Australia, the species is restricted to rainforest mountain-tops within the Wet Tropics region. Individuals were collected from two distinct populations in the Australian Wet Tropics from March 2016 to June 2016 to provide replication within species and between populations. Flies were collected from a population located within Paluma National Park (19° 0'16.27"S, 146°12'35.59"E) and a population located approximately 400 km north within Mt Lewis National Park (16°35'30.36"S, 145°16'27.78"E; Fig. 5.2B). Flies were collected directly from banana baits (method described in Higgie & Blows, 2008) and isofemale lines were started by first identifying individuals to the species level and subsequently placing a single, wild-caught, gravid *D. birchii* female in a vial to lay. If females had not already mated in the wild, they were placed with a wild-caught male from the same population to mate. Flies were then transported back to the laboratory and maintained in isofemale lines at 23°C in a controlled environment room under 12 hr light: dark cycles. Flies were reared on standard Drosophila food that contained sugar, yeast, and agar as described in Higgie & Blows (2008).

Creation of heatwave treatment populations

I created three heatwave treatment populations from each of the wild populations of *D. birchii*. To do so, I first assessed the performance of twelve isofemale lines from each wild population using a static heat knockdown assay to assess heat knockdown time (KD_T). I chose to perform static heat knockdown assays as a proxy to a heatwave as they create extremely stressful thermal conditions for individuals which are also experienced during a heatwave.

Isofemale lines were maintained in the laboratory under controlled conditions for 18 generations before the assay, and were maintained in large population numbers to limit genetic drift and inbreeding and to maintain the underlying genetic architecture of each wild-caught female. The generation before the heat knockdown assay, twenty virgin females and twenty virgin males from each isofemale line were sexed and five female: male pairs were placed in four individual 300 mL bottles. Each bottle contained 100 mL *Drosophila* food sprinkled with live yeast to promote ovipositing. Flies were allowed to mate for 48 hrs at

23°C in 12 hr light: dark cycles. By limiting the mating time, I was able to control for density and limit negative effects caused by larval competition. Bottles were then left in controlled conditions and flies were allowed to develop. Mating bottles were cleared of flies at the first sign of eclosing and left for 12 hrs, after which all adult flies were collected and sexed under low anaesthetic (via CO_2). Females from each replicate bottle were collected and placed in vials at five females per vial with 10 mL food. Females were allowed to recover for four days before the start of the assay to reduce dehydration effects caused by anaesthetisation and allow for sexual maturation.

To perform the static heat knockdown assay, individual flies were placed in empty vials in a temperature chamber (equipment described in Greenspan et al., 2016) at 39°C and watched until flies were immobilized and movement ceased. The time when movement in the wings stopped was recorded as KD_T. Ten flies were placed in the incubator during each run and all flies were scored by the same watcher to limit bias. Flies were scored blind by randomly placing them within a vial marked one to ten within the incubator. Five females per bottle were measured for each isofemale line (with four founding bottles), resulting in twenty females assayed per isofemale line for each wild population. Isofemale line replicates and populations were randomized within and across runs to limit confounding effects of run and account for any variance in the performance of the temperature chamber across runs. In addition, vial position within the incubator was recorded and included in statistical models to account for any potential difference in temperature within the chamber. Temperature was measured at two spots within the incubator for quality control.

Mean KD_T was calculated for each isofemale line and lines were bred to create populations with differing thermal tolerances. The heatwave treatment populations included one control population bred from isofemale lines with the five highest and five lowest mean thermal tolerances, one population bred from offspring of the isofemale lines with the five highest mean thermal tolerances, and one population bred from offspring of the isofemale line that exhibited the highest mean thermal tolerance. Each artificial population was founded from the same number of individuals (180 individuals with 50:50 female to male ratio) by initially mating one female and one male from each relevant line (and vice versa, with numerous replicates) and randomly collecting offspring from these crosses and placing into three replicate 300 mL bottles with 100 mL *Drosophila* food. All subsequent generations were maintained from these mass bred populations.

Heritability analysis

I performed a variance component analysis to calculate the narrow-sense heritability of KD_T for each wild population. The variance attributed to each factor for each study population was estimated using restricted maximum likelihood (REML) and *P*-values were obtained by comparing linear mixed effect models with and without the factor of interest using ANOVA. Models included run as a random factor with isofemale line and within-line replicate as nested random factors. All factors were treated as random intercept terms because an intercept only model is necessary to determine the variance around factors (Zuur et al., 2009; Harrison et al., 2018). 95% confidence intervals on variance were also calculated to check whether there was significant variance explained by each factor. Within-line variance was estimated as the variance of replicates and residuals. Between-line variance was estimated from the variance of lines. Heritability was calculated by dividing the additive genetic variance (V_A) by the total variance (V_{Total}); with V_A calculated as the variance between lines ($V_{between}$) divided by two times the inbreeding coefficient and isofemale heritability estimated as the variance between lines ($V_{between}$) divided by the sum of the variance between ($V_{between}$) and variance within lines (V_{within}). The package lme4 (Bates et al., 2020) was used in the statistical program R (R Core Team, 2019) to carry out model estimation and comparison.

Second heat knockdown assay

Treatment populations were maintained at large sizes ($N > 1000$) in a controlled laboratory for the span of time from creation to the start of this experiment and to our knowledge no selection was placed upon them. Approximately one year after I created the heatwave treatment populations, I subjected them to a second extreme heat event (‘heatwave 2’) per the heat knockdown assay method described above. 100 females per population and treatment ($N = 200$ per treatment) were assayed from twenty founding bottles over two blocks (performed from two succeeding generations). All populations and replicates were randomized across runs and blocks.

Thermal performance curve measurements

I used adjustable temperature arrays (as described in Cocciardi et al., 2019) to create the thermal resource gradient where each temperature point was assigned a unique temperature and a 100 mL vial with 10 mL *Drosophila* food was placed in each. Each population was

randomly assigned a temperature array and fitness measures were collected from four replicates in a randomized complete block design, where each population was represented in each block. Temperature arrays were set up in two controlled environment rooms at 25°C and 65% RH and relative humidity within vials was 85%–90%. Linear thermal-gradients were set up along the temperature points measuring 20°–36°C, in 1°C intervals and the direction of the gradient was randomized across temperature arrays. 10 μg of a diluted water: baker yeast solution (10: 1 parts) was deposited on top of *Drosophila* food to promote ovipositing and all food vials were randomized across temperature arrays.

One generation before the start of the experiment, density-controlled mass bred bottles were created from each treatment by breeding 30 females and 30 males each, in three 300 mL bottles with 100 mL standard *Drosophila* food. This was done to control for larval density within the bottle and limit competition effects, as well as control for body size of offspring which were used as parents in the subsequent experiment. Additionally, to confirm parent body size was consistent across the experiment, three female parents were randomly collected from each replicate after mating and the left wing was measured per methods described in Hoffmann & Shirriffs (2002). Parent wing size was compared across treatments to confirm no significant variation was introduced from parent body size ($R^2 = 0.005$, $F_{2,192} = 1.54$, $P = 0.217$).

Parents were sexed as virgin flies from the density-controlled bottles and placed in low-density (5 individuals per vial) holding vials that contained 10 mL standard *Drosophila* food. Flies were held for 3–4 days before the start of the experiment to ensure sexual maturity; after which, one female and two males (to ensure mating occurred) were placed within each stoppered vial at each temperature point and left for 48 hours before being removed. All vials were carded with pupation card at 72 hrs and offspring were allowed to develop. Starting at day five, vials were checked every 24 hr for pupae and emerging adults. If adults had emerged within the previous 24 hr period, they were collected and placed in holding vials before being sexed and counted. Each block ran for 17 days, which allowed for the maximum development time at the coolest and hottest temperature used in this thermal gradient (per observations by J. Cocciardi) to allow for full development of all possible offspring.

Relative fitness measures

Total productivity was calculated by summing the total offspring that emerged from each vial. Average offspring development speed was calculated by taking the inverse of the average 24 hr period that adults emerged from each vial. In addition, a randomly selected group of females were frozen from each emergence day from every vial (if available). At the end of the experiment, three females per temperature per replicate were randomly selected from these (using a random number generator) and the left wing was wing-mounted and landmarks were digitized to obtain offspring body size for each vial as per method described in Chapter 2 of this thesis and in Hoffmann & Shirriffs (2002). This method takes the landmarked coordinates for each wing and computes the square root of the summed squared distance between a centroid configuration and all landmarks. This results in one value called the centroid size that provides a measure of overall size and is expressed in arbitrary units (Rohlf & Slice, 1990; Rohlf, 2000).

Statistical analysis

Data exploration for all analyses were carried out following the protocol described in Zuur et al. (2010) and all subsequent analyses were performed using the statistical program R (R Core Team, 2019). Outliers were identified by 1.5 interquartile range and removed before all analyses.

Second extreme heat event

I compared the knockdown times of heatwave treatment populations from the second heat knockdown assay ('heatwave 2') by modelling KD_T of heatwave treatment as a function of heatwave treatment type using a Gamma GLMM with a log link function. The log link function ensures positive fitted values, and the Gamma distribution is used for continuous data with greater spread in the data and is the distribution that fits this dataset. A potential interaction between heatwave treatment and wild population was removed due to non-significance, as was the fixed covariate of wild population. Block was removed from the model due to collinearity with the covariate run. In this case, it is recommended to keep the factor that accounts for the majority of the variation (Zuur et al., 2009; Harrison et al., 2018), so run remained in the model to account for time differences between runs and bocks. To incorporate the potential dependency of individuals collected from the same mass bred, mass

bred was treated as a random intercept term, as were vial position and run. Random slopes were investigated for all random intercept terms but removed due to the resulting model converging and being over-fitted. Model assumptions were verified by plotting standardized versus fitted residuals and versus all covariates.

Thermal performance curve and reactions norms

To compare productivity between populations, a zero-inflated negative binomial GLMM with a log link function was modelled using the package *glmmTMB* (Brooks et al., 2017) in R. Total offspring was modelled as a function of heatwave treatment type, wild population, and temperature. Temperature was scaled and centred and modelled as a quadratic due to the typical pattern of total offspring decreasing on either side of T_{opt} in a TPC. An interaction between treatment and population was found to be significant and was included. A potential interaction between treatment and both the linear and quadratic temperature variable was insignificant and dropped from the model. To incorporate the potential dependency among individuals from the same mass bred, replicate was included as a random intercept and slope term nested within treatment type. All model assumptions were verified using the *DHARMa* package (Hartig & Lohse, 2020) in R by plotting bootstrapped residuals against predicted values and all covariates (Appendix D Figure 4). All tests of uniformity, zero-inflation, dispersion, and independence were verified.

Both development speed and wing size were compared across treatment types using a linear mixed model fit in the package *nlme* (Pinheiro et al., 2020) in R. Development speed and centroid size were modelled as a function of treatment type and temperature, with replicate as a random intercept term nested within treatment to account for potential dependency of individuals from the same mass bred and generation. Temperature was scaled and centred and modelled as a quadratic and interaction terms between both temperature variables and treatment were included in the model for wing size but found to be insignificant and dropped from the final model for development speed. The number of total offspring that emerged from each vial was initially included as a random-intercept term, but dropped from the final model for both traits as it was found to be insignificant. It was initially included because total offspring is expected to vary unequally by temperature and the amount of offspring that develop within a vial may create competition that can affect development time and body size. Final models for both traits were obtained by comparing the full, null model with a nested model using maximum likelihood. The final model was fit using REML. All

model assumptions were verified by plotting normalized residuals against predicted values and all covariates (Appendix D Figures 5, 6). Tests of uniformity, zero-inflation, dispersion, and outliers were also verified.

Parameters for each TPC and reaction norm were obtained by fitting pre-defined functions to each treatment and population's dataset using non-linear least square models. All functions were fit using the *nlstools* (Baty et al., 2015) and *rTPC* (Padfield & O'Sullivan, 2020) package in R. Because the Gaussian distribution is typical of TPCs (Angilletta, 2006), several of the functions tested consisted of modified-Gaussian fits (Appendix D Table 6). Productivity curves were modelled by weighing each observation by its variance due to the overall heteroskedastic nature of the data (Appendix D Figure 1). Parameters were directly estimated from the functions and CT_{max} was estimated as the highest temperature at which performance was 5% the maximum performance (P_{max}) because model functions often result in the curve never reaching zero or predicting zero at temperatures that have little biological meaning (Kellermann et al., 2019). 95% confidence intervals were calculated for each curve by using first-order Taylor expansion and Monte Carlo simulation (K = 100,000) using the function 'predictNLS' in the R-package *propagate* (Spiess, 2018).

In addition to the functions that productivity was fit to, development speed and wing size were also fit to a quadratic, cubic and quartic function (Appendix D Table 6). Development speed and wing size models were not weighted as there was homoscedasticity across observations and because some variables were missing replicates which would result in skewed weighing (Appendix D Figures 2, 3). All model fits were assessed visually and the best fit model was chosen as the model with the lowest AICc score. If models had a difference in AICc score of less than 2, then model parameters were weighted and the averaged parameter was used. Parameters were compared by using a one-way ANOVA with treatment as the grouping variable.

5.6 Highlights

- I investigated how one heatwave affects the long-term thermal tolerance and fitness of impacted populations after several generations of benign conditions.
- I found that one hard selection event did not induce adaptation in thermal-stress tolerance, but did cause maladaptation; most likely due to decreased genetic diversity in impacted populations.
- We cannot presume heatwave survivors will be better adapted to future heatwaves; an alarming conclusion given climate change predictions.

Chapter 6: General Discussion

Global warming due to climate change is impacting biodiversity, and impacts are predicted to be profound over the coming decades. Impacts include range shifts, changes to behaviour and breeding biology, altered communities, population declines, and extinctions (Thomas et al., 2004; Thuiller et al., 2004; Franks et al., 2007; Berg et al., 2010; Bellard et al., 2012; Franks et al., 2016; Pecl et al., 2017). Many species will have to adapt to the changing conditions to survive under increasing global temperatures (Hoffmann & Sgrò, 2011; Huey et al., 2012). Key characteristics of a species' thermal niche will determine their potential for adaptation (Huey et al., 2012) and may impose physiological limits that determine their vulnerability to climate change (Hoffmann, 2010).

In ectotherms, the thermal niche is controlled by characteristics such as thermal tolerance, thermal performance, and thermoregulatory behaviour (Fig. 6.1). The thermal niche is most often described using thermal tolerances and thermal performance, but rarely thermoregulatory behaviour. A meta-analysis on the term 'thermal niche' (Gvoždík, 2018) found that 47% of studies describe the thermal niche using indirect measurements of thermal tolerance from distributional data, 36% use thermal performance measurements, 10% directly measure thermal tolerances, and 7% use thermoregulatory behaviour. Although any one of these components can describe a portion of a species' thermal niche, a more comprehensive approach would be to incorporate all three facets into the thermal niche descriptor. Generally, this thesis investigates the interaction of thermal tolerances, thermal performance, and thermal behaviour of two closely-related *Drosophila* species (Fig. 6.1).

More specifically, this thesis considers how each facet of a specialist and generalist species' thermal niches were affected by environmental change. Williams et al. (2008) laid out an integrative framework for researchers investigating the capacity of a species to survive under climate change. This framework states that a species vulnerability to environmental changes and their evolutionary trajectory will depend on the sensitivity and genetic make-up of thermal performances, thermal tolerances, and thermoregulatory behaviour. This is including, but not limited to: 1) how increasing temperatures affect important fitness traits, 2) whether important fitness traits can adapt to novel conditions, 3) how thermoregulatory behaviour affects adaptation potential of important fitness traits, and 4) whether heat-tolerance traits are able to adapt and how this affects long-term fitness. Collectively, each

chapter of this thesis provides information on one of these aspects to assess adaptation under climate change in a well-studied *Drosophila* pair (*Drosophila birchii* and *D. serrata*).

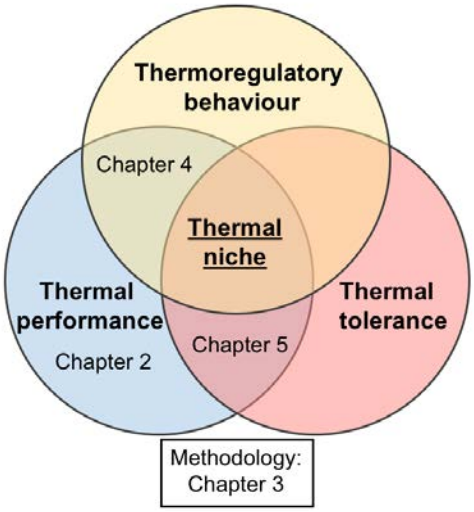

Figure 6.1: Key components of an ectotherm's thermal niche.
A species' thermal niche is defined as the thermal space where population growth occurs. In the absence of biotic interactions, an ectotherm's thermal niche will mainly be determined by the interaction of its thermoregulatory behaviour, thermal performances, and thermal tolerances (Gvoždík, 2018).

In chapter 2, I examined thermal performance in key fitness traits under a benign and stressful thermal environment and found temperature stress can alter the heritability, additive genetic variance, and evolvability of fitness traits in an inconsistent way. In Chapter 3, I described equipment that I designed for the purpose of investigating broad ecological and evolutionary questions on thermal tolerances, thermal performances, and thermoregulatory behaviour. This equipment was subsequently used to examine how thermal behaviour, measured as oviposition temperature preference, relates to the optimal performance temperature of key fitness traits in a generalist and a specialist species (Chapter 4). This thesis showed that an ultimate fitness trait is tightly coadapted to temperature preference. Lastly, I examined how a heatwave affects long-term thermal tolerances and thermal performances of key fitness traits (Chapter 5) and found that directional selection on thermal tolerance did not increase mean thermal tolerances but, instead, caused maladaptation in heatwave-survivor populations.

6.1 Thermal performance: genetic variance and covariances

This thesis provided evidence that an important life history trait (fecundity) has greater evolvability under a stressful environment than under a benign environment for two species of closely-related *Drosophila.* This bodes well for the species examined here, both of which are found in tropical ecosystems—where gradual warming may push species past their current thermal tolerances, so adaptation will most likely be necessary (Kingsolver et al., 2013). Incorporating adaptive genetic variation into climate change models is essential to accurately predicting species vulnerability. For example, adaptive genetic variation was incorporated into distribution changes under climate change scenarios for two species of bats and the results showed that accounting for additive genetic variance resulted in less predicted range-loss when compared to not incorporating additive genetic variance (Razgour et al., 2019).

Historically, environmentally-dependent genetic variance has been well-studied because it is a key first step to understanding how environmental change affects adaptation potential (Rowiński & Rogell, 2017; Fischer et al., 2020). However, no trend has been detected; potentially because many studies focus on heritability rather than direct changes to additive genetic variance (Fischer et al., 2020). Heritability is sensitive to residual variation (including environmental variation), meaning comparisons between environments cannot be made (Houle, 1992; Hoffmann & Merilä, 1999; Rowiński & Rogell, 2017; Fischer et al., 2020). This thesis presented a prime example of why heritability values are important to understanding the absolute change in a trait mean from one generation under selection to the next; but heritability may only tell a portion of the story when examining the evolutionary potential of a trait across environments.

For example, it has generally been assumed that life history traits exhibit low heritability due to decreased additive genetic variance (Mousseau & Roff, 1987; Falconer & Mackay, 1996; Merilä & Sheldon, 1999). Here, I showed that heritability for a life history trait (fecundity) is low in *D. birchii* and *D. serrata*, which coincides with previous research (Gilchrist & Partridge, 1999; Hoffmann & Shirriffs, 2002; Moraes et al., 2004; Kellermann et al., 2006). However, by explicitly measuring standardized metrics of additive genetic variance (i.e., the coefficient of additive genetic variance and evolvability), I showed that additive genetic variance in fecundity is actually very high. Instead of reduced additive genetic variance, an increased residual variance seems to be the main driver behind a low heritability value for fecundity in these species. This is important because it shows how

heritability values, that are standardized by total genetic variance, can be misleading when comparing individual components of genetic variance. Studies should additionally examine standardized additive genetic variance values that are more appropriate for comparisons across traits because they are standardized by the trait mean (Houle, 1992). This is because, as shown in this thesis, these values may indicate that a trait thought to have low heritability may still exhibit high additive genetic variance; and that this could be a result of increased non-additive genetic variance that is driving heritability down.

The results found here, that fecundity exhibits a low heritability but high additive genetic variance, coincide with a previous study on the collared flycatcher (Merilä & Sheldon, 2000) and a general review on available datasets (Merilä & Sheldon, 1999) that showed fitness traits generally exhibit a high residual variance due to non-additive genetic variance (i.e., increased environmental variance, dominance variance and epistatic variance) and early-development environmental effects. Additionally, Merilä and Sheldon (1999) found that residual variance in morphological traits is reduced compared to life history traits; consistent with what I found here in wing size. Overall, my results coincided with this review to provide additional evidence that the type of trait, as well as the environment, will influence the genetic architecture of a trait.

Another reason why there has been no discernible trend detected in how genetic variance changes across environments is that genetic variance values are specific to species and populations and are sensitive to even slight environmental changes (as evidence by changes between generations). Here, I found no consistent trend as to how genetic variance changed between thermal environments, closely-related *Drosophila* species, or populations within each species. More specifically, when comparing the results presented here and a similar study (Kellermann et al., 2006), it seems that even a 1°C or 2°C difference in thermal environment will affect the expression of genetic variance. Generally, this could be caused by a decreased trait mean that would produce a larger percentage change while still resulting in a similar absolute change—but in this case, it is specifically due to an increased additive variance. Investigation into how different magnitudes of change affect genetic variance would help to identify if a threshold exists—where additive genetic variance can be assumed to be the same under the threshold but not over. For example, if additive genetic variance for a trait changed between environments that differed by 1°C, but did not significantly change in environments that differed by 0.5°C or 0.1°C; then this knowledge can be incorporated into evolutionary trajectories.

A link between the trait phenotypes assessed here and fitness in the wild will also aid in interpreting these results in an evolutionary framework (Werf et al., 2009). For example, although variation in morphological traits and the genes responsible for them is well studied in *Drosophila*, there are few studies quantifying how these morphological traits influence fitness in the field (Hine et al., 2004; Werf et al., 2009).

Perhaps the greatest consideration in a study like the one presented here is that traits are not individually selected for in the wild. Real-world fitness is fundamentally multivariate (Lande & Arnold, 1983; Catullo et al., 2019), meaning both fitness and morphological traits will be selected for in nature. Hence, determining whether genetic correlations exist between life history and morphological traits is important. If fitness is positively correlated to a morphological trait under selection, adaptation will occur and this will promote species survival in a novel environment. If the opposite is true, then selection on fitness may constrain evolution in the other trait (and vice versa; Conner, 2012; Wood & Brodie, 2016). For example, selection on desiccation resistance in *D. melanogaster* led to correlated changes in body size and fecundity in some experiments but not others (Hoffmann & Parsons, 1988b; Rose et al., 1992; Bubliy & Loeschcke, 2005; Telonis-Scott et al., 2006; Tejeda et al., 2016)—emphasizing that genetic correlations are also environmentally-dependent. Here, I also investigated genetic correlations between fecundity and wing size in different environments. Although previous research has shown a high positive phenotypic and genetic correlation between wing length and fecundity in *Drosophila* (Chiang & Hodson, 1950; Tantawy & Vetukhiv, 1960; Santos et al., 1992; Woods et al., 2002), other studies have not found this relationship (Woods et al., 2002; Sgrò & Hoffmann, 1998b). This thesis also found inconsistent phenotypic and genetic correlations between fecundity and wing morphometric traits across environments—which is most likely due to environmental variation and/or environment-specific gene effects that are masking phenotypic correlations.

6.2 Thermoregulatory behaviour and thermal performance

Theory predicts that the thermal optimum for fitness should correlate with the temperature preference of a species, in order to maximize fitness at temperatures experienced in the wild (known as the ‘thermal coadaptation hypothesis; (Huey & Bennett, 1987; Huey & Kingsolver, 1989; Angilletta, 2009). This thesis confirmed that an ultimate fitness trait is tightly coadapted to temperature preference in two closely-related species, providing support for the thermal coadaptation hypothesis. Additionally, I tested two competing hypotheses

describing different methods of coadaptation between temperature preference and thermal performance in a thermal generalist versus a thermal specialist species. I did not find supporting evidence for either hypothesis but instead I provide evidence that suggests temperature preference range is confined around the thermal optimum in a thermal generalist and thermal specialist species, regardless of thermoregulatory behaviour and performance strategy.

This is important because thermoregulatory behaviour, which is controlled by temperature preference in ectotherms (Angilletta, 2009), is thought to promote and/or hinder adaptation to changing conditions (Angilletta, 2009; Dillon et al., 2009; Buckley et al., 2015). Specifically, thermoregulatory behaviour may 'conserve' thermal performance because individuals will use behaviour to move to preferred microclimates and this will reduce selection from novel environmental conditions (i.e., the 'Bogert effect'; Bogert, 1949; Huey et al., 2003; and see Buckley et al., 2015 for example). Species that use thermoregulatory behaviour as a 'buffer' to climate change may potentially have an increased risk of extinction because of this (Huey & Kingsolver, 1993; Huey et al., 2003; Kearney et al., 2009; Buckley et al., 2015). Hence, understanding the relationship between thermoregulatory behaviour and performance may help to inform researchers how temperature preferences will affect adaptation. For example, if temperature preference is currently located above the thermal performance optima for key fitness traits and the environmental temperature increases, thermoregulatory behaviour may promote adaptation. However, the more common prediction is that preferred temperatures sit below the thermal optimum (Martin & Huey, 2008; Huey et al., 2012) and that climate warming will cause temperature preferences to increase. This will result in either a temperature preference closer to the thermal optimum (which will increase fitness) or a temperature preference above the thermal optimum (which will decrease fitness; see Fig. 1 in Huey et al., 2012).

Here, I found no trend as to whether temperature preference is located above or below thermal optima. Rather, I found that the location of temperature preference in relation to the thermal optimum (above or below) was conserved across species within each trait. This is relevant because the species studied here are sister-species, so this result may indicate that the selective pressure for temperature preference to coadapt with each trait was the same for both species regardless of localized selection imposed by the different environments they reside in. Incorporating phylogeny, as well as additional data from other closely-related species, would inform us whether a more concrete trend exists in where temperature preference is located in comparison to peak thermal performance for specific types of traits. Temperature preference

could then be used as a proxy for thermal performance in species that thermal stress tests cannot be performed on, such as rare or threatened species. For example, I show that temperature preference is highly correlated to productivity (a trait directly related to reproductive success), and not coadapted with development speed and wing size. If researchers are interested in the temperature where optimum fitness occurs, temperature preference may be a good indicator. However, if researchers are looking for temperatures that maximize a certain developmental or morphological trait, temperature preference may provide an optimum temperature range but may not provide a precise prediction.

Whether temperature preference is heritable and under what conditions also needs to be considered. Temperature preference has been found to exhibit a high heritability in some species of *Drosophila* (see Dillon et al., 2009 for review), and a low heritability in others (Castañeda et al., 2019). Understanding whether temperature preference is coadapted to certain fitness traits through behaviour or through genetic covariances or gene-environment effects would shed light on the mechanisms of coadaptation. It may be possible that both are true (temperature preference has coadapted to certain thermal optimums from behaviour and from genetic linkages), but understanding the multifaceted aspects of temperature preference and performance is fundamental to understanding a species thermal niche.

Additionally, investigating the range of temperature preferences within a population is important because a population that exhibits a narrow temperature preference range (a thermal specialist) may have more trouble adapting to novel conditions than a species that exhibits a wider temperature preference range (a thermal generalist; Buckley et al. 2015). In any case, if temperature preference can adapt will depend on the amount of additive genetic variance a population exhibits in temperature preference (Sinervo et al., 2010; Buckley et al., 2015). Plasticity in temperature preference has also been relatively overlooked and could provide an extra buffer to climate change in many species (Gvoždík, 2012). However, plasticity in temperature preference may detrimentally affect adaptation potential by decreasing selection on both thermal tolerances and thermal behaviour, or it may aid adaptation if the ability to be plastic in temperature preference is being selected for.

6.3 Thermal tolerance and thermal performance

This thesis provides evidence that heat-induced mortality from a heatwave may not always increase the thermal tolerance of a population, and hence populations may not be better adapted to surviving a second heatwave. In addition, the results suggest that maladaptation to

subsequent heat events can occur, and this is likely caused by decreased genetic diversity in small populations that survive heatwaves.

Loss of genetic diversity will directly affect adaptation potential to novel environments and may cause additional detrimental effects from increased inbreeding depression (e.g., Coleman et al., 2020; Gurgel et al., 2020). Reduced genetic diversity may be the most serious consequence of an extreme heat event, but is often overlooked because it is not always evident from examining phenotypes alone (Gurgel et al., 2020). This leads to what is termed 'cryptic' genetic diversity loss. Additionally, decreased genetic diversity may also lead to detrimental performance effects, such as not being able to compete or predate adequately (Sanz-Lázaro, 2016; Zhang et al., 2016; Grant et al., 2017). Empirical research into how whole communities are affected by heatwaves needs to be conducted, in addition to empirical studies into the adverse effects of heatwaves on genetic diversity in the wild, which are currently scarce (Gurgel et al., 2020).

Furthermore, how selection on thermal tolerance affects thermoregulatory behaviour should also be considered. This thesis has shown that thermoregulatory behaviour is coadapted to thermal performance and that thermal performance is adversely affected by hard selection on thermal tolerance—so a relationship between acute thermal tolerances and thermoregulatory behaviour also needs to be considered. If the two are genetically linked or correlated then selection on one will affect the other. This is important because many ectotherms have a very small thermal 'safety margin' (i.e., the difference between the environmental temperature and a species' maximum thermal tolerance limit), so any warming in environmental temperature will be detrimental to species without the ability to regulate behaviourally (e.g., Sunday et al., 2014). However, if selection on thermal tolerances also causes adaptation in thermoregulatory behaviour, then species should exhibit increased adaptation potential.

An important consideration when examining the results from this chapter is that thermal tolerance evolution may be influenced by the methodology employed (Terblanche et al., 2007; Sgrò et al., 2010; Rezende et al., 2011; Terblanche et al., 2011; Kingsolver & Umbanhowar, 2018). For example, some studies use static tests of thermal tolerance and other studies use dynamic, ramping tests. Static tests include those such as static knockdown assays that test the immediate response of an individual to a stressful temperature. Conversely, ramping knockdown assays test the response of an individual to acclimate more slowly to temperatures that rise gradually over several hours. Initially, ramping assays were thought to be more ecologically relevant because extreme temperatures in nature will usually

occur over several hours to several days (Terblanche et al., 2007; Overgaard et al., 2011). However, recent studies have found that ramping assays may cause a higher level of heat stress due to associated factors such as increased dehydration and resource depletion. Because of this, the shorter, high-temperature static assays are thought to provide a better estimate of the adaptive potential of heat tolerance (Blackburn et al., 2014; van Heerwaarden & Sgro, 2014; Castañeda et al., 2019). Overall, results from static assays usually depict a higher evolutionary potential for the trait being measured than ramping assays (Blackburn et al., 2014; van Heerwaarden & Sgro, 2014; Castañeda et al., 2019), and provide a more precise estimate of the genetic component of heat tolerance traits (Rezende et al., 2011; Santos et al., 2011; Castañeda et al., 2019). Hence in this thesis, I used static heat knockdown assays to predict the adaptive potential of heat tolerance in several populations. However, research into how a static versus ramping assay affects thermal performance measures would further this field. Specifically, investigating the difference in how thermal tolerance selection imposed by static and ramping assays affects long-term thermal tolerance and performance can provide insight into how the onset of extreme events (an acute onset or a slow onset) affects population's thermal tolerances, thermal performance, and thermal behaviour.

An additional consideration on the potential effects of heatwaves in the wild is that a population's current thermal tolerance may not correlate perfectly to their current distribution because of biotic constraints on their fundamental niche (i.e., the realized niche)—meaning their thermal tolerances may extend into other environments (Bocsi et al., 2016; Catullo et al., 2019; Razgour et al., 2019). This would mean that directional selection from a heatwave or other extreme weather events would not occur and certain populations may not be affected by extreme weather events at all (Catullo et al., 2019).

6.4 Conclusion

When taken together, the results presented here show that thermal performance is related to thermoregulatory behaviour and that both can be directly affected by changing temperatures and changing thermal tolerances. These results give a synthesized view of the thermal niche of two closely-related *Drosophila* species. However, how thermal performance, thermal behaviour, and thermal tolerance will respond to increasing environmental temperatures, and whether adaptation of each facet will occur, will also be dictated by biotic interactions. For example, a theoretical study found that incorporating high levels of intraspecific competition into evolutionary rescue models lowered abundance, and decreased genetic diversity and the

rate of beneficial mutations (e.g., Osmond & de Mazancourt, 2012). Conversely, interspecific competition aided evolutionary rescue by pushing adaptation to occur at a more rapid rate. Competition, predation, parasitism, and diseases must be considered in the evolutionary framework when determining adaptation potential (Buckley & Roughgarden, 2006; Sinervo et al., 2010; Harley, 2011).

More generally, other factors such as demographic processes and gene flow may limit adaptation potential. Specifically, it's been shown that the vulnerability of *D. birchii* to heat stress will depend heavily on demographic factors when population size is less than 20, will depend on both genetic and demographic factors when the population is around 100, and will not experience detrimental effects when the population in greater than 1000 (e.g., Willi & Hoffmann, 2009). Gene flow may also hinder adaptation when there is a large spill-over of alleles from the centre of a population to its range edges (e.g., Bridle & Vines, 2007), and this has been shown to occur along the southern geographical range of *D. serrata* (e.g., Magiafoglou et al., 2002).

A last consideration is that plasticity of thermal performance, thermal tolerance traits, and thermoregulatory behaviour also needs to be considered in the context of adaptation (Catullo et al., 2019). Phenotypic plasticity is often thought to help an individual survive changing environmental conditions (i.e., plastic rescue; Chevin et al., 2012; Kelly, 2019), but can also hinder adaptation by shifting phenotypes in the opposite direction of selection (Catullo et al., 2019; Fox et al., 2019).

This thesis emphasizes that predicting the adaptive response of a species to climate change will be challenging because the thermal niche is a multifaceted and complicated property. However, climate change is one of the greatest threats to biodiversity and continued detailed research that recognises the complexity of the thermal niche is required to determine whether adaptation will see species through this challenge.

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Appendix A: Supporting information for Chapter 2

Appendix A Table 1. **Sample sizes, mean trait values, phenotypic variances (V_P), additive variances (V_A), residual variances (V_{res}), coefficient of additive variance (CV_A), and evolvabilities (I_A) for fitness as measured by fecundity in a benign (23ºC) and stressful (28ºC) environment.**

Standard errors for CV_A were calculated by adding and subtracting the SE of heritability from the heritability value and then calculated V_A from each estimate (i.e., $V_A = h^2 \times V_P$) to obtain an approximate lower standard error (*LSE*) and upper standard error (*USE*).

Fecundity

				23°C (Benign environment)					
Species	Generation	*N*	*mean* ± *SE* (no. of offspring)	V_P	V_A	V_{res}	$CV_A \times 10^2$	*SE* CV_A *x 10 [LSE; USE]*	$I_A \times 10^2$
D. birchii	Pooled	179	90.1 ± 2.78	1384.6	204.921	1179.679	15.888	[7.39; 21.22]	2.524
	Dams	91	101.5 ± 2.60	630.4	-	-	-	-	-
	Daughters	88	78.4 ± 4.70	1906.3	-	-	-	-	-
D. serrata	Pooled	154	144.19 ± 2.67	1105.1	57.465	1047.635	5.257	[0; 9.67]	0.276
	Dams	81	131.2 ± 3.50	991.4	-	-	-	-	-
	Daughters	73	158.7 ± 3.40	843.4	-	-	-	-	-
				28°C (Stressful environment)					
D. birchii	Pooled	-	-	-	-	-	-	-	-
	Dams	86	16.20 ± 1.86	299.8	-	-	-	-	-
	Daughters	-	-	-	-	-	-	-	-
D. serrata	Pooled	152	85.34 ± 3.51	1879.5	75.180	1804.320	10.165	[0; 21.50]	1.032
	Dams	84	76.8 ± 4.30	1548.1	-	-	-	-	-
	Daughters	68	95.9 ± 5.60	2111.7	-	-	-	-	-

Appendix A Table 2. **Sample sizes, mean trait values, phenotypic variances (V_P), additive variances (V_A), residual variances (V_{res}), coefficient of additive variance (CV_A), and evolvabilities (I_A) for wing size in a benign (23ºC) and stressful (28ºC) environment**.

Wing size is shown as the log centroid size, which produces an arbitrary unit of measurement for comparison purposes. Standard errors for CV_A were calculated by adding and subtracting the SE of heritability from the heritability value and then calculated V_A from each estimate (i.e., $V_A = h^2 \times V_P$) to obtain an approximate lower standard error (*LSE*) and upper standard error (*USE*).

Wing size

			23°C (Benign environment)						
Species	Generation	*N*	*mean* ± *SE* (log centroid size)	V_P	V_A	V_{res}	$CV_A \times 10^2$	*SE* $CV_A \times 10$ *[LSE; USE]*	$I_A \times 10^2$
D. birchii	Pooled	173	6.8786 ± 0.0016	0.0005	0.0002	0.0003	0.224	[0.191; 0.253]	0.001
	Dams	86	6.8813 ± 0.0026	0.0006	-	-	-	-	-
	Daughters	87	6.8758 ± 0.0020	0.0004	-	-	-	-	-
D. serrata	Pooled	145	6.9076 ± 0.0020	0.0005	0.0005	0.0000	0.324	[0.299; 0.346]	0.001
	Dams	79	6.9065 ± 0.0024	0.0004	-	-	-	-	-
	Daughters	66	6.9088 ± 0.0034	0.0007	-	-	-	-	-
			28°C (Stressful environment)						
D. birchii	Pooled	-	-	-	-	-	-	-	-
	Dams	78	6.7723 ± 0.0026	0.0005	-	-	-	-	-
	Daughters	-	-	-	-	-	-	-	-
D. serrata	Pooled	147	6.8159 ± 0.0019	0.0005	0.0001	0.0004	0.156	[0.105; 0.194]	0.0002
	Dams	81	6.8091 ± 0.0026	0.0005	-	-	-	-	-
	Daughters	66	6.8244 ± 0.0025	0.0004	-	-	-	-	-

Appendix A Table 3. Sample sizes, mean trait values, heritabilities (h^2), phenotypic variances (V_P), additive variances (V_A), and residual variances (V_{res}) for the relative warp (RW) parameters for wing shape in a benign (23ºC) and stressful (28ºC) environment.

Heritabilities shown in bold are significantly different from zero and the asterisks indicate the significance level for adjusted *P*-values (adjusted by False Discovery Rate; **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001). The percentage of variation that each RW score accounts for is also shown.

Wing shape

				23°C (Benign environment)					
Species		Generation	*N*	*mean* ± *SE*	h^2	V_P	V_A	V_{res}	% variation
D. birchii	RWb-1	Pooled	173	0.0061 ± 0.0008	**0.412** ± 0.22*	1.12E-04	4.66E-05	6.58E-05	34.47
		Dams	86	0.0069 ± 0.0011	-	1.12E-04	-	-	-
		Daughters	87	0.0053 ±0.0010	-	8.94E-05	-	-	-
	RWb-2	Pooled	173	0.0005 ± 0.0007	**0.356** ± 0.22**	1.12E-04	3.05E-05	8.12E-05	16.97
		Dams	86	0.0017 ± 0.0011	-	7.21E-05	-	-	-
		Daughters	87	-0.0008 ± 0.0008	-	5.78E-05	-	-	-
	RWb-3	Pooled	173	-0.001 ± 0.0007	**0.680** ± 0.22***	7.21E-05	4.90E-05	2.31E-05	13.09
		Dams	86	-0.009 ± 0.0010	-	7.21E-05	-	-	-
		Daughters	87	-0.0011 ±0.0008	-	5.33E-05	-	-	-
	RWb-4	Pooled	164	0.0007 ± 0.0005	0.142 ± 0.22	3.73E-05	5.30E-06	3.20E-05	6.54
		Dams	86	0.0011 ± 0.0007	-	3.73E-05	-	-	-
		Daughters	87	0.0003 ± 0.0006	-	3.03E-05	-	-	-
	RWb-5	Pooled	173	-0.0002 ± 0.0004	**0.457** ± 0.22*	3.31E-05	1.51E-05	1.80E-05	6.07
		Dams	86	-0.00004 ± 0.0006	-	3.58E-05	-	-	-
		Daughters	87	-0.0003 ± 0.0006	-	3.07E-05	-	-	-
	RWb-6	Pooled	173	0.0003 ± 0.0004	0.035 ± 0.22	2.62E-05	6.89E-10	2.62E-05	5.55
		Dams	86	0.0005 ± 0.0006	-	2.75E-05	-	-	-
		Daughters	87	0.00003 ± 0.0005	-	2.52E-05	-	-	-
D. serrata	RWs-1	Pooled	145	0.0116 ± 0.0009	**1.00** ± 0.25****	1.05E-04	1.05E-04	0.00E+00	41.11
		Dams	79	0.010 ± 0.0010	-	1.04E-04	-	-	-
		Daughters	66	0.013 ± 0.0010	-	1.02E-04	-	-	-
	RWs-2	Pooled	145	0.0002 ± 0.0007	**0.836** ± 0.25*	7.56E-05	6.32E-05	1.24E-05	15.96
		Dams	79	0.0002 ± 0.0090	-	6.63E-05	-	-	-
		Daughters	66	0.0003 ± 0.0110	-	8.77E-05	-	-	-
	RWs-3	Pooled	145	-0.0017 ± 0.0006	**1.00** ± 0.25****	5.79E-05	5.79E-05	0.00E+00	10.27
		Dams	79	-0.0025 ± 0.0008	-	5.62E-05	-	-	-
		Daughters	66	-0.008 ± 0.0009	-	5.90E-05	-	-	-
	RWs-4	Pooled	145	-0.0004 ± 0.0005	0.514 ± 0.25	4.01E-05	2.06E-05	1.95E-05	7.24
		Dams	79	-0.0011 ± 0.0006	-	3.00E-05	-	-	-
		Daughters	66	0.0005 ± 0.0009	-	5.12E-05	-	-	-
	RWs-5	Pooled	145	0.0008 ± 0.0005	0.510 ± 0.25	3.58E-05	1.82E-05	1.75E-05	6.45
		Dams	79	0.0008 ± 0.0007	-	3.96E-05	-	-	-
		Daughters	66	0.0008 ± 0.0005	-	3.58E-05	-	-	-

28°C (Stressful environment)

Species		Generation	*N*	*mean ± SE*	h^2	V_P	V_A	V_{res}	% variation
D. birchii	RWb-1	Pooled	-	-	-	-	-	-	34.47
		Dams	78	-0.0135 ± 0.0011	-	9.41E-05	-	-	-
		Daughters	-	-	-	-	-	-	-
	RWb-2	Pooled	-	-	-	-	-	-	16.97
		Dams	78	-0.001 ± 0.0011	-	9.63E-05	-	-	-
		Daughters	-	-	-	-	-	-	-
	RWb-3	Pooled	-	-	-	-	-	-	13.09
		Dams	78	0.0022 ± 0.0008	-	5.50E-05	-	-	-
		Daughters	-	-	-	-	-	-	-
	RWb-4	Pooled	-	-	-	-	-	-	6.54
		Dams	78	-0.0016 ± 0.0006	-	2.45E-05	-	-	-
		Daughters	-	-	-	-	-	-	-
	RWb-5	Pooled	-	-	-	-	-	-	6.07
		Dams	78	0.0004 ± 0.0006	-	2.93E-05	-	-	-
		Daughters	-	-	-	-	-	-	-
	RWb-6	Pooled	-	-	-	-	-	-	5.55
		Dams	78	-0.0006 ± 0.0007	-	3.55E-05	-	-	-
		Daughters	-	-	-	-	-	-	-
D. serrata	RWs-1	Pooled	147	-0.0116 ± 0.0009	**0.268** ±0.25**	1.06E-04	2.84E-05	7.76E-05	41.11
		Dams	81	-0.015 ± 0.001	-	1.14E-04	-	-	-
		Daughters	66	-0.008 ± 0.001	-	7.11E-05	-	-	-
	RWs-2	Pooled	147	-0.0002 ± 0.0009	**0.668** ±0.25*	1.12E-04	7.45E-05	3.70E-05	15.96
		Dams	81	-0.0015 ± 0.0012	-	1.19E-04	-	-	-
		Daughters	66	0.0014 ± 0.0012	-	1.00E-04	-	-	-
	RWs-3	Pooled	147	0.0017 ± 0.0006	0.576 ±0.25	5.67E-05	3.27E-05	2.40E-05	10.27
		Dams	81	0.0024 ± 0.0008	-	4.82E-05	-	-	-
		Daughters	66	0.0009 ± 0.0010	-	6.69E-05	-	-	-
	RWs-4	Pooled	147	0.0004 ± 0.0006	0.414 ±0.25	4.45E-05	1.84E-05	2.61E-05	7.24
		Dams	81	0.00001 ± 0.0008	-	5.00E-05	-	-	-
		Daughters	66	0.0008 ± 0.0008	-	3.81E-05	-	-	-
	RWs-5	Pooled	147	-0.0008 ± 0.0005	0.430 ±0.25	3.86E-05	1.66E-05	2.20E-05	6.45
		Dams	81	0.00001 ± 0.0007	-	3.91E-05	-	-	-
		Daughters	66	-0.0018 ± 0.0007	-	3.66E-05	-	-	-

Appendix A Table 4: Phenotypic correlations between traits in *D. birchii* and *D. serrata* reared under two temperatures.

r_P is the phenotypic correlation and the *P*-value is adjusted by False Discovery Rate and was obtained from a *F*-test of the linear regression of one trait on the other. Bold values indicate significance and significance level is shown in asterisks (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). In addition, a *F*-test was conducted to determine if the interaction of population with this regression was significant, which tests whether the phenotypic correlation varies between the two populations of a species. Where there was a significant interaction, r_P was estimated for each population individually.

Trait 1 ~ Trait 2	Species	Trait	Generation Population	r_P	*SE*	*P*-value	r_P	*SE*	*P*-value
				Benign (23°C)			**Stressful (28°C)**		
Fecundity ~ Wing size	*D. birchii*		Dams	0.30	0.108	0.437	0.22	0.117	0.647
			Mt. Lewis	-0.14	0.147	0.763	-	-	-
			Paluma	0.76	0.147	0.104	-	-	-
			Daughters	0.58	0.103	0.073	-	-	-
	D. serrata		Dams	-0.14	0.117	0.760	-0.18	0.117	0.707
			Daughters	0.12	0.123	0.763	0.52	0.125	0.202
Fecundity ~ Wing shape	*D. birchii*	RWb-1	Dams	-0.22	0.108	0.625	-	-	-
			Daughters	0.10	0.108	0.763	-	-	-
		RWb-2	Dams	0.10	0.109	0.763	-	-	-
			Daughters	0.20	0.108	0.647	-	-	-
		RWb-3	Dams	0.06	0.109	0.834	-	-	-
			Daughters	-0.26	0.108	0.507	-	-	-
		RWb-4	Dams	-0.20	0.109	0.647	-	-	-
			Mt Lewis	-0.76	-	0.104	-	-	-
			Paluma	0.04	-	0.507	-	-	-
			Daughters	-0.16	0.108	0.707	-	-	-
		RWb-5	Dams	0.16	0.109	0.707	-	-	-
			Daughters	-0.40	0.106	0.243	-	-	-
		RWb-6	Dams	-0.10	0.109	0.763	-	-	-
			Daughters	0.42	0.106	0.218	-	-	-
	D. serrata	RWs-1	Dams	0.10	0.115	0.763	0.24	0.11	0.625
			Daughters	0.08	0.126	0.837	-0.22	0.11	0.647
		RWs-2	Dams	-0.16	0.115	0.707	0.10	0.11	0.787
			Daughters	0.04	0.126	0.902	-0.52	0.12	0.203
		RWs-3	Dams	0.10	0.115	0.763	0.42	0.11	0.247
			Daughters	-0.04	0.125	0.902	0.06	0.13	0.856
		RWs-4	Dams	0.10	0.115	0.763	0.26	0.11	0.581
			Daughters	-0.14	0.125	0.763	0.54	0.12	0.202
		RWs-5	Dams	0.16	0.115	0.760	-0.40	0.11	0.283
			Daughters	0.36	0.125	0.437	0.48	0.12	0.218
			Mt Lewis	-	-	-	**0.98***	-	0.049
			Paluma	-	-	-	-0.06	-	0.897
Wing size ~ Wing shape	*D. birchii*		Dams	0.26	-	0.437	0.33	-	0.218
			Daughters	0.39	-	0.065	-	-	-
	D. serrata		Dams	0.43	-	0.052	0.28	-	0.401
			Daughters	**0.59***	-	0.049	0.28	-	0.565

Appendix A Table 5: Genetic covariances and correlations between traits in *D. birchii* and *D. serrata* reared under a benign (23ºC) and stressful (28 ºC) environment.

Genetic covariances and correlations were calculated in both directions, meaning one trait in the dam was regressed on the other trait in the daughters and vice versa. *N* indicates the number of family pairs used in the regression, the slope for the regression is indicated by β, the genetic covariance of one trait on the other is cov_{XY}, the covariances for the individual traits are shown as cov_{XX} and cov_{YY}, and the genetic correlation (r_G) was calculated using the equation set forth in Falconer and Mackay (1996). The adjusted *P*-value for the regression is noted (adjusted by False Discovery Rate), as well as the *P*-value for a *F*-test on the interaction. between population and the trait value ($P\text{-value}_{Pop}$). If population was significant, individual parameters were estimated for each. Genetic correlations shown in the paper were calculated from the mean of the genetic covariances in both directions.

Species	Dam - daughter Population	Benign (23ºC)							
		N	β	cov_{XY}	cov_{XX}	cov_{YY}	r_G	*P*-value	$P\text{-value}_{pop}$
D. birchii	Fecundity–Wing size	85	0.14	0.280	0.148	0.476	1.05	0.869	0.863
	Wing size–Fecundity	82	-0.09	-0.180	0.476	0.148	-0.68	0.869	0.335
	Fecundity–RWb-1	85	-0.07	-0.140	0.148	0.462	-0.54	0.869	0.407
	RWb-1–Fecundity	82	0.03	0.060	0.462	0.148	0.23	0.932	0.282
	Fecundity–RWb-2	85	0.07	0.140	0.148	0.356	0.61	0.869	0.354
	RWb-2–Fecundity	82	-0.03	-0.060	0.356	0.148	-0.26	0.932	0.671
	Fecundity–RWb-3	85	-0.13	-0.260	0.148	0.680	-0.82	0.869	0.867
	RWb-3–Fecundity	82	0.03	0.060	0.680	0.148	0.19	0.932	0.485
	Fecundity–RWb-4	85	-0.07	-0.140	0.148	0.284	-0.68	0.869	0.141
	RWb-4–Fecundity	82	0.02	0.040	0.284	0.148	0.20	0.960	0.273
	Fecundity–RWb-5	85	0.06	0.120	0.148	0.457	0.46	0.876	0.013
	Mt Lewis	40	0.32	0.640	0.148	0.457	2.46	0.490	-
	Paluma	45	-0.21	-0.420	0.148	0.457	-1.61	0.869	-
	RWb-5–Fecundity	82	-0.04	-0.080	0.457	0.148	-0.31	0.932	0.658
	Fecundity–RWb-6	85	-0.19	-0.380	0.148	0.035	-5.28	0.655	0.683
	RWb-6–Fecundity	82	-0.13	-0.260	0.035	0.148	-3.61	0.869	0.592
	Wing Size–RWb-1	81	0.08	0.160	0.476	0.462	0.34	0.869	0.759
	RWb-1–Wing Size	81	-0.04	-0.080	0.462	0.476	-0.17	0.932	0.293
	Wing Size–RWb-2	81	0.12	0.240	0.476	0.356	0.58	0.869	0.218
	RWb-2–Wing Size	81	0.07	0.140	0.356	0.476	0.34	0.869	0.327
	Wing Size–RWb-3	81	-0.07	-0.140	0.476	0.680	-0.25	0.869	0.579
	RWb-3–Wing Size	81	0.07	0.140	0.680	0.476	0.25	0.869	0.980
	Wing Size–RWb-4	81	-0.03	-0.060	0.476	0.284	-0.16	0.932	0.734
	RWb-4–Wing Size	81	0.03	0.060	0.284	0.476	0.16	0.932	0.156
	Wing Size–RWb-5	81	0.07	0.140	0.476	0.457	0.30	0.869	0.297
	RWb-5–Wing Size	81	-0.08	-0.160	0.457	0.476	-0.34	0.869	0.993
	Wing Size–RWb-6	81	-0.03	-0.060	0.476	0.035	-0.46	0.932	0.663
	RWb-6–Wing Size	81	0.02	0.040	0.035	0.476	0.31	0.960	0.643

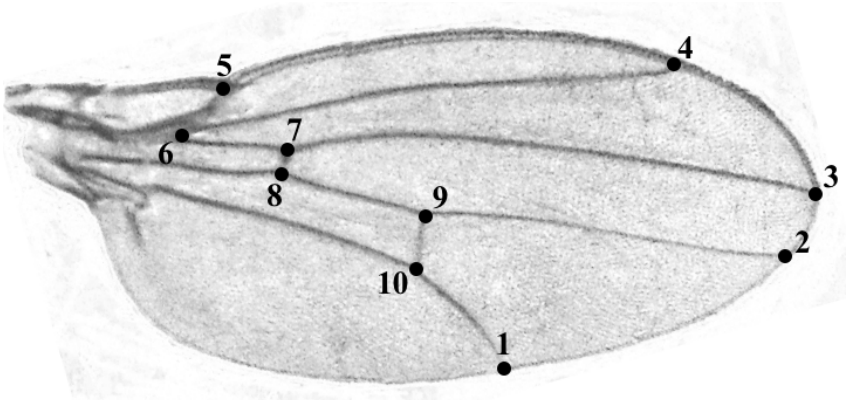
Species	Dam - daughter Population	Benign (23ºC)							
		N	β	cov_{XY}	cov_{XX}	cov_{YY}	r_G	*P*-value	*P*-value$_{pop}$
D. serrata	Fecundity–Wing size	63	-0.08	-0.160	0.052	1.000	-0.70	0.932	0.292
	Wing size–Fecundity	70	-0.16	-0.320	1.000	0.052	-1.40	0.869	0.078
	Fecundity–RWs-1	63	0.11	0.220	0.052	1.000	0.96	0.869	0.736
	RWs-1–Fecundity	86	0.06	0.120	1.000	0.052	0.53	0.932	0.821
	Fecundity–RWs-2	63	-0.09	-0.180	0.052	0.836	-0.86	0.869	0.832
	RWs-2–Fecundity	68	0.08	0.160	0.836	0.052	0.77	0.869	0.001
	Paluma	34	0.42	0.840	0.836	0.052	4.03	0.234	-
	Raglan Ck	34	-0.36	-0.720	0.836	0.052	-3.45	0.455	-
	Fecundity–RWs-3	63	0.1	0.200	0.052	1.000	0.88	0.869	0.857
	RWs-3–Fecundity	68	0.29	0.580	1.000	0.052	2.54	0.234	0.905
	Fecundity–RWs-4	63	-0.12	-0.240	0.052	0.514	-1.47	0.869	0.934
	RWs-4–Fecundity	68	-0.03	-0.060	0.514	0.052	-0.37	0.932	0.469
	Fecundity–RWs-5	63	0.11	0.220	0.052	0.514	1.35	0.869	0.511
	RWs-5–Fecundity	68	0.12	0.240	0.052	0.510	1.47	0.869	0.106
	Wing Size–RWs-1	63	0.01	0.020	1.000	1.000	0.02	0.981	0.630
	RWs-1–Wing Size	62	0.17	0.340	1.000	1.000	0.34	0.869	0.364
	Wing Size–RWs-2	63	0.19	0.380	1.000	0.836	0.42	0.869	0.771
	RWs-2–Wing Size	62	-0.04	-0.080	0.836	1.000	-0.09	0.932	0.631
	Wing Size–RWs-3	63	0.08	0.160	1.000	1.000	0.16	0.869	0.421
	RWs-3–Wing Size	62	0.13	0.260	1.000	1.000	0.26	0.869	0.667
	Wing Size–RWs-4	63	0.17	0.340	1.000	0.514	0.47	0.869	0.896
	RWs-4–Wing Size	62	0.01	0.020	0.514	1.000	0.03	0.981	0.296
	Wing Size–RWs-5	63	-0.15	-0.300	1.000	0.510	-0.42	0.869	0.076
	RWs-5–Wing Size	62	0.1	0.200	0.510	1.000	0.28	0.869	0.790

Species	Dam - daughter Population	Stressful (28 ºC)							
		N	β	cov_{XY}	cov_{XX}	cov_{YY}	r_G	*P*-value	*P*-value$_{pop}$
D. serrata	Fecundity–Wing size	63	0.02	0.040	0.040	0.226	0.42	0.981	0.715
	Wing size–Fecundity	64	0.06	0.120	0.226	0.040	1.26	0.944	0.769
	Fecundity–RWs-1	63	0.05	0.100	0.040	0.268	0.97	0.932	0.609
	RWs-1–Fecundity	64	-0.02	-0.040	0.268	0.040	-0.39	0.960	0.641
	Fecundity–RWs-2	63	0.12	0.240	0.040	0.668	1.47	0.869	0.149
	RWs-2–Fecundity	64	0.08	0.160	0.668	0.040	0.98	0.869	0.217
	Fecundity–RWs-3	63	-0.01	-0.020	0.040	0.576	-0.13	0.981	0.504
	RWs-3–Fecundity	64	-0.08	-0.160	0.576	0.040	-1.05	0.869	0.850
	Fecundity–RWs-4	63	0.36	0.720	0.040	0.414	5.60	0.164	0.057
	RWs-4–Fecundity	64	-0.22	-0.440	0.414	0.040	-3.42	0.651	0.331
	Paluma	26	0.05	0.100	0.414	0.040	0.78	0.932	-
	Raglan Ck	38	-0.46	-0.920	0.414	0.040	-7.15	0.164	-
	Fecundity–RWs-5	63	0.01	0.020	0.040	0.430	0.15	0.981	0.183
	RWs-5–Fecundity	64	0.00	0.000	0.430	0.040	0.00	0.983	0.293
	Wing Size–RWs-1	62	-0.31	-0.620	0.226	0.268	-2.52	0.234	0.419
	RWs-1–Wing Size	62	-0.09	-0.180	0.268	0.226	-0.73	0.869	0.748
	Wing Size–RWs-2	62	0.05	0.100	0.226	0.668	0.26	0.932	0.518
	RWs-2–Wing Size	62	-0.06	-0.120	0.668	0.226	-0.31	0.932	0.171
	Wing Size–RWs-3	62	-0.13	-0.260	0.226	0.576	-0.72	0.869	0.908
	RWs-3–Wing Size	62	0.11	0.220	0.576	0.226	0.61	0.869	0.823
	Wing Size–RWs-4	62	0.07	0.140	0.226	0.414	0.46	0.876	0.035
	Paluma	26	0.38	0.760	0.226	0.414	2.48	0.512	-
	Raglan Ck	36	-0.16	-0.320	0.226	0.414	-1.05	0.869	-
	RWs-4–Wing Size	62	-0.10	-0.200	0.414	0.226	-0.65	0.869	0.366
	Wing Size–RWs-5	62	0.18	0.360	0.226	0.430	1.15	0.869	0.227
	RWs-5–Wing Size	62	0.10	0.200	0.430	0.226	0.64	0.869	0.917

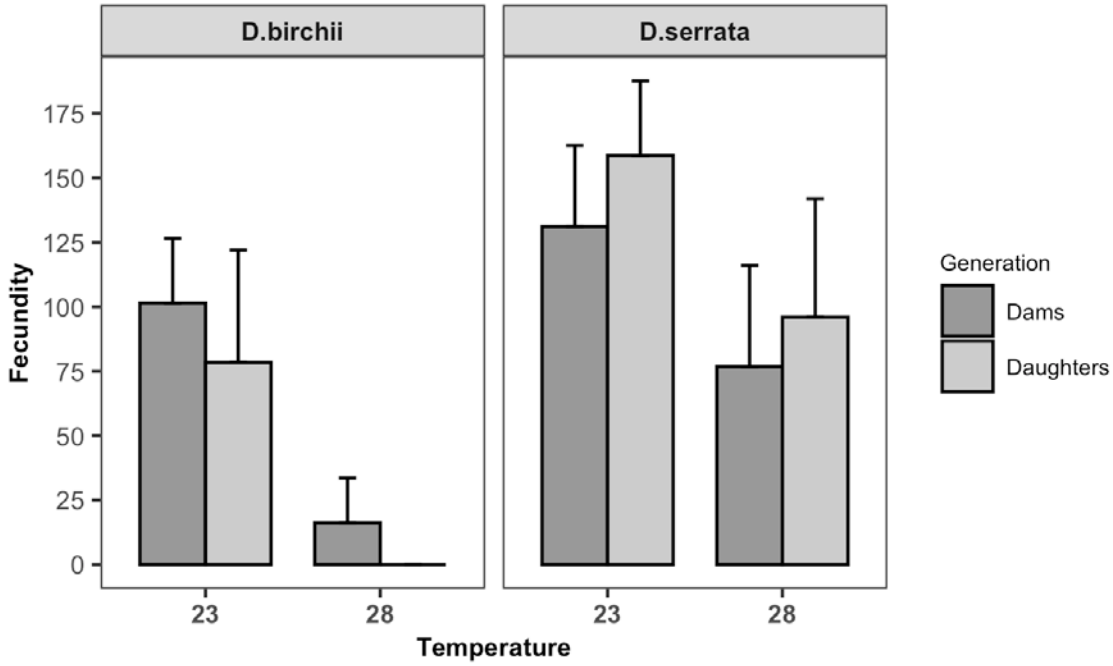
Appendix A Table 6: **Genetic correlations between fitness, wing size, and wing shape in two different thermal environments**.

r_G denotes the genetic correlation between trait one ($trait_1$) and trait two ($trait_2$) and *SE* is the standard error for the genetic correlation. Wing shape variables are shown as the relative warp (RW) scores that contribute to > 5% variability. Bold values indicate a statistically significant correlation based on standard errors. *P*-values were adjusted for False Discovery Rate and significance level is shown with asterisks (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001).

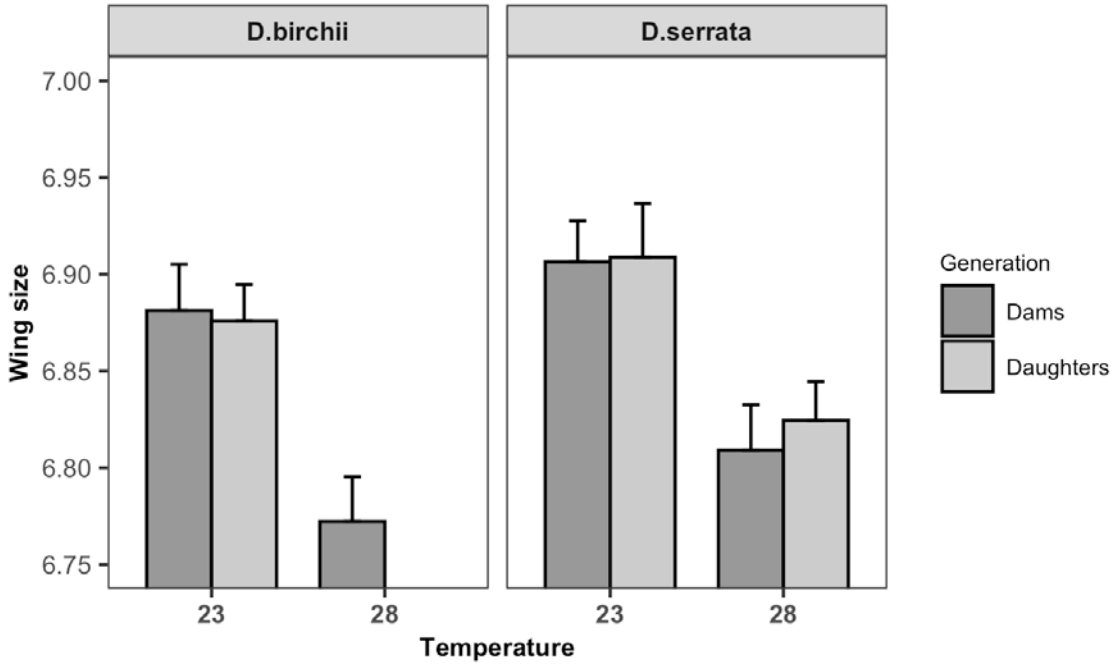
			Benign (23ºC)			**Stressful (28 ºC)**		
Species	$trait_1$	$trait_2$	r_G	*SE*	*P*-value	r_G	*SE*	*P*-value
D. birchii	Fecundity	Wing size	0.19	0.32	1.000	-	-	-
	Fecundity	RWb-1	-0.15	0.11	1.000	-	-	-
	Fecundity	RWb-2	0.17	0.28	1.000	-	-	-
	Fecundity	RWb-3	-0.32	0.19	1.000	-	-	-
	Fecundity	RWb-4	-0.24	0.39	1.000	-	-	-
	Fecundity	RWb-5	0.08	0.29	1.000	-	-	-
	Fecundity	RWb-6	0.00	0.00	0.000	-	-	-
	Wing size	RWb-1	0.09	0.07	0.500	-	-	-
	Wing size	RWb-2	**0.46**	0.14	0.006**	-	-	-
	Wing size	RWb-3	0.00	0.13	1.000	-	-	-
	Wing size	RWb-4	0.00	0.25	1.000	-	-	-
	Wing size	RWb-5	-0.02	0.17	1.000	-	-	-
	Wing size	RWb-6	-0.08	0.21	1.000	-	-	-
D. serrata	Fecundity	Wing size	**-1.00**	0.04	6.96E-120****	0.84	0.29	0.019*
	Fecundity	RWs-1	**0.75**	0.16	2.60E-05****	0.29	0.76	1.000
	Fecundity	RWs-2	-0.05	0.45	1.000	1.00	0.27	0.166
	Fecundity	RWs-3	1.00	0.73	1.000	-0.59	0.43	1.000
	Fecundity	RWs-4	**-0.92**	0.10	4.37E-12****	**1.00**	0.13	4.44E-08****
	Fecundity	RWs-5	1.00	0.49	1.000	0.08	0.71	1.000
	Wing size	RWs-1	0.18	0.08	0.094	-1.00	0.54	0.203
	Wing size	RWs-2	0.16	0.11	0.394	-0.03	0.22	1.000
	Wing size	RWs-3	0.21	0.09	0.085	-0.06	0.27	1.000
	Wing size	RWs-4	0.25	0.14	0.215	-0.10	0.28	1.000
	Wing size	RWs-5	-0.07	0.12	1.000	**0.90**	0.06	8.36E-45****



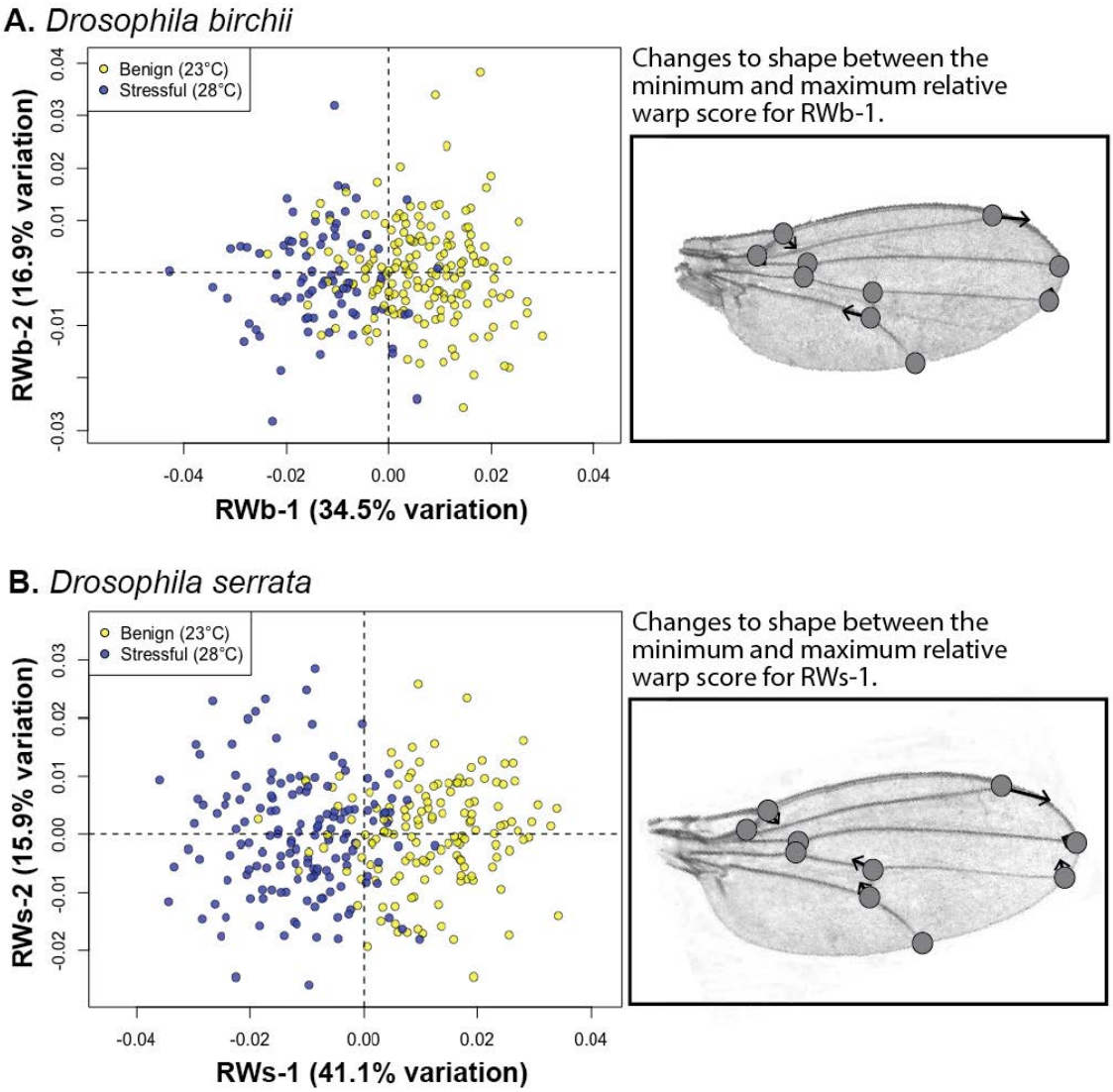
Appendix A Figure 1: An example of a wing image, showing the 10 landmark positions in sequential order that were used to compute centroid size for wing size and relative warp scores for wing shape.



Appendix A Figure 2: Fecundity of dams and their daughters exposed to two different thermal environments for the entirety of their life. Fecundity is based on total egg count of 72 hrs. Error bars show the standard deviations and means and sample sizes are shown in Appendix A Table 1.



Appendix A Figure 3: Wing size of dams and their daughters exposed to two different thermal environments for the entirety of their life. Wing size is shown as log centroid size that is measured in arbitrary units. Error bars show the standard deviations and means and sample sizes are shown in Appendix A Table 2.



Appendix A Figure 4: PCA plots showing the RW 1 and RW 2 axes for wing shape in (a) *Drosophila birchii* and (b) *D. serrata* grouped by temperature treatment and for all individuals measured (i.e., both dams and daughters). Enlarged images of wings show the directionality and position (indicated by the black arrows) of change to each landmark between the minimum (shown) and maximum (end of the arrow) relative warp score for RWb/s-1. Means and samples sizes are shown in Appendix A Table 3.

Appendix B: Supporting information for Chapter 3

Appendix B Supplementary Material 1: Costing and retailer information for parts needed to make one adjustable temperature array (ATA).

ATA part	Components	Retailer	Price/part	Quantity	Cost
Temperature strip	**Temperature Strip: *Temperature points***				
	Baseboard	Local hardware store	$5.00	1	$5.00
	Aluminium water-cooled heat-exchange bars	Online retailer	$12.00	3	$36.00
	7 W thermoelectric Peltier heat pump (-55°C to 88°C)	Online retailer	$1.00	18	$18.00
	Aluminium machined vial holders	Local hardware store and machined at University workshop	$10.00	1	$10.00
	DS1820 temperature sensor	Local electronics store or online retailer	$0.50	18	$9.00
	16-pin female plug-socket cable	Online retailer	$3.00	3	$9.00
	Temperature Strip: *Water-cooling system*				
	Aluminium heatsink bars	As above	-	-	-
	6 mm plastic tubing	Local hardware store	$1.40/1m	1	$1.40
	Irrigation manifolds	Local hardware store	$1.00	1	$1.00
	Water feature/pond pump + tubing (optional)	Local hardware store	$50.00	1 for entire system	$50.00
	Hose clamps	Local hardware store	$0.25	2	$0.50
Control box	**Control Box: *Electronics***				
	16-channel 12-bit PWM servo drivers (i.e., switching solid-state relays)	Local electronics store or online retailer	$2.00	3	$6.00
	Dual-channel H-bridge motor driver shields (i.e., switching solid-state relays)	Local electronics store or online retailer	$2.00	18	$36.00
	16-pin male plug-socket cables	Local electronics store or online retailer	$3.00	3	$9.00
	Solderless breadboard jumper cable wires	Local electronics store or online retailer	$10.00/100	1	$10.00
	Control Box: *Microcontroller*				
	a) WeMoS-D1R2 microcontroller (i.e., embedded microcontroller)	Local electronics store or online retailer	$7.00	1	$7.00
	b) 400 kW resistors	Local electronics store or online retailer	$0.50	2	$1.00
	Control Box: *LED Lighting System*				
	a) WS2812 RGB LED module string	Local electronics store or online retailer	$5.00	1	$5.00
	b) 400 kW Ohm 0.5W metal film resistors	Local electronics store or online retailer	$0.50	3	$1.50
Cage	**Experimental setup: *Cages***				
	a) 34 L clear storage containers	Local hardware store	$8.00	1	$8.00
	b) Pantyhose	Local grocery or department store	$2.00	1	$2.00
TOTAL COST OF 1 ATA UNIT:					**$175.40**

Notes: The water feature is in grey as only one is needed per water-cooling system. This cost is not included in the total cost for 1 ATA unit.

Appendix B Supplementary material 2: Design and construction of the temperature strip components.

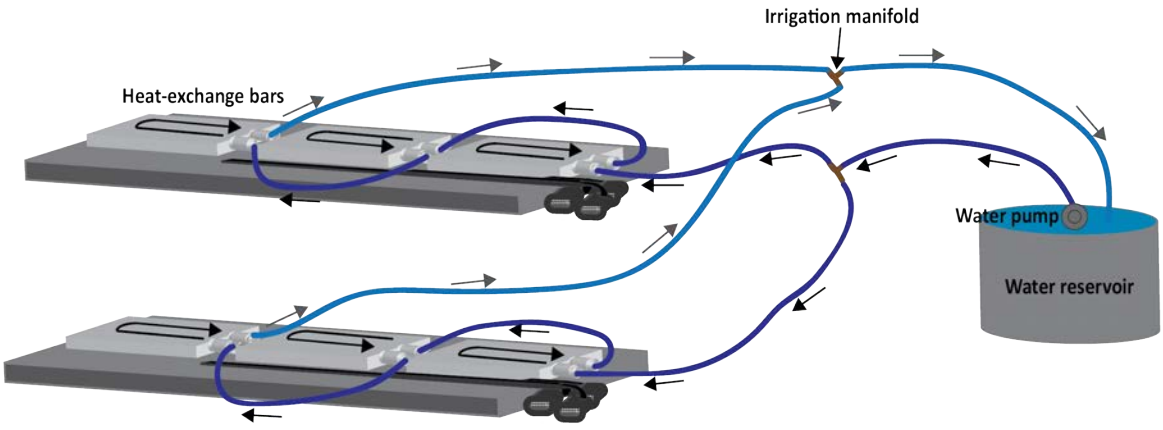
Temperature strip

The components used to create 18 temperature points are listed in Table 1 and shown in Fig. 1 of the main manuscript. We chose to use aluminium for parts due to its high conductivity. To construct the temperature strip, the aluminium heatsink bars were mounted end to end onto the stable baseboard. Next, each temperature point was made by gluing a heat pump to the aluminium heatsink bars and then an aluminium vial holder to each heat pump (Fig. 1a) using a silicone thermal conductive glue. Each heat pump contains a positive and negative feedback wire, which were soldered to the 16-pin plug socket cable (Fig. 1b).

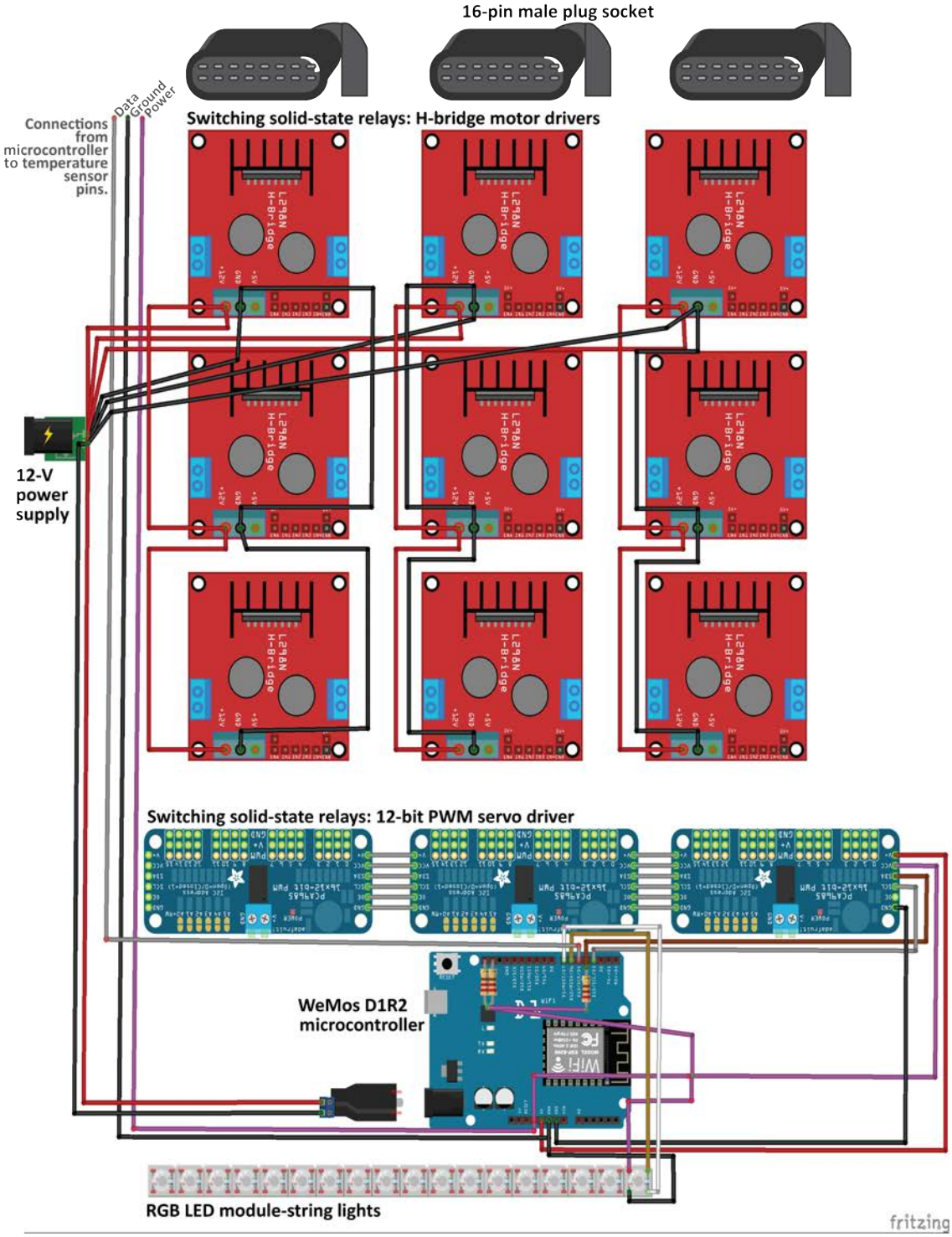
Once assembled, a single temperature sensor was super-glued to each vial-holder (Fig 1c). Each temperature sensor contains three pins (power, data, and ground). All pins must be connected to the microcontroller to allow for data collection and over/under temperature feedback to control the specified temperatures. To do this, a copper wire was soldered in parallel to the temperature sensor power pins at each chamber using a daisy-chain. The data and ground pins were then daisy-chained in the same way with their own copper wires. The three copper wires were then connected to the 16-pin plug-socket cable to provide real-time temperature measurements for each temperature point (Fig. 1b).

Water-cooling system

The water-cooling system uses a cold-water reservoir that pumps water through aluminium heatsink bars to redistribute and strip excess heat away from the temperature strips and is necessary for the heat pumps' performance. The water-cooling system can be attached to a constantly flowing cold water tap (i.e., mains water) or a simple water pump can be used to cycle water through the system if the user can keep the reservoir water cold. The heat-exchange bars have a U-shaped hollow with 6 mm connections to allow water to flow through and strip away excess heat. Here, we attached the 'in-flow' tubing for each ATA to each other via a T-junction irrigation manifold, which was connected to the cold-water tap. We then connected the 'out-flow' tubing for each ATA to each other to allow for heated water leaving heat-exchange bars to be dispensed back to the reservoir area (Appendix B Figure 2.1).



Appendix B Figure 2.1: The design of the water system is shown above, with black arrows indicating the 'in-flow' of cool water from the water reservoir to and throughout the heat-exchange bars. Grey arrows indicate the 'out-flow' of water that has stripped excess heat from the heat-exchange bars and is returning to the reservoir to be cooled.



Appendix B Supplementary Material 3: The components of the control box are shown above. The set up and placement of parts inside the control box and the wiring needed to connect the electronic components can be seen in the schematic. Both solderless breadboard jumper cable wires and standard hook-up wires were used.

Appendix B Supplementary Material 4: LED lights correspond to each temperature point and change based on the following equation, where warm colours (red–red-yellow) indicate heating response to deviance while cold colours (blue–blue-green) indicate a cooling response. This allows for quick indication of the temperature of a specific temperature point.

Colour	**Display**	**Actual temperature deviance from target temperature (t_T)**
Red	Flashing	$-5°C < t_T$
Red	Solid	-5 to $-1°C < t_T$
Red-yellow	Solid	-1 to $-0.5°C < t_T$
Green	Solid	$-0.5°C < t_T < 0.5°C$
Blue-green	Solid	0.5 to $1°C > t_T$
Blue	Solid	1 to $5°C > t_T$
Blue	Flashing	$5°C > t_T$

Appendix B Supplementary Material 5: Additional data and results.

Validation tests: temperature deviations

Please refer to Appendix B Figures 5.1, 5.2 below for additional plots showing temperature deviations in actual temperatures to temperature set-points for each of the four thermal landscapes examined in the validation tests.

Temperature variation within vials: methods and results

Methods

We tested how temperatures varied within vials by setting up one ATA with a closed vial design and one ATA with an open cage design in a controlled temperature room at 23°C and 50% RH. Chambers within each ATA were randomly assigned to one of three testing temperatures (15°, 25°, or 36°C) and randomly assigned to have a resource present or to not have a resource present. Data loggers (Thermochron DS1921G. Maxi3m Integrated. San Jose, CA) were placed at the bottom and top of each vial and set to record every minute for 24 hrs. Data loggers at the bottom of each vial were placed either on 5 mL Drosophila yeast-agar-sugar mix resource (i.e., 1 cm from the bottom) or on no resource (0.5 cm from the bottom of the vial). Data loggers at the top were taped to the inside of the vial at 2 cm from the top of the vial. Foam stoppers were then placed in the vials above the data loggers for the closed vial setup. A cage was placed on top of vials for the open cage setup. Each treatment (open caged–resource, open caged–no resource, closed vial–resource, closed vial–no resource) was tested at each temperature three times.

Results

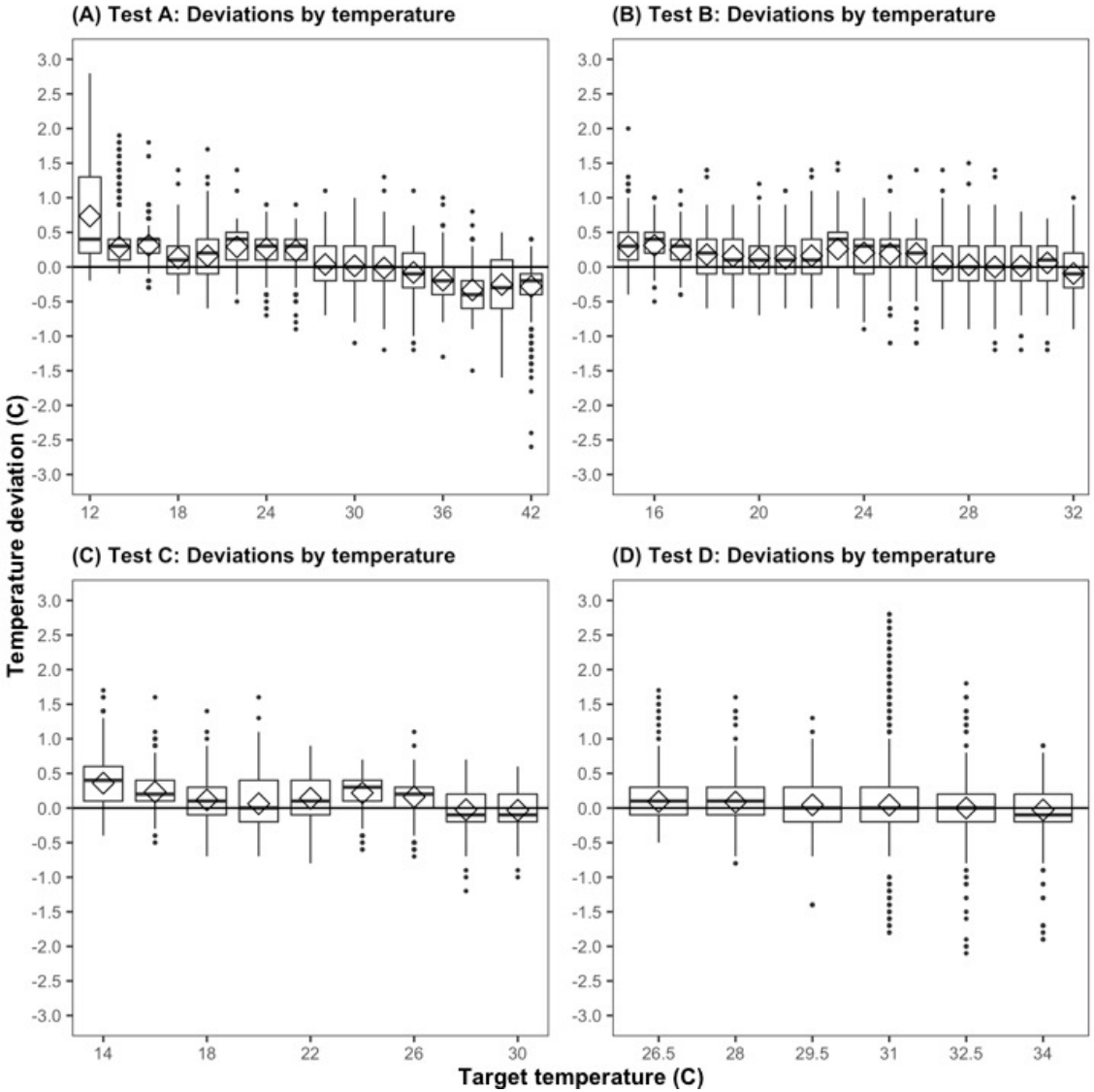
Results are shown below in Appendix B Table 5.1 and Figures 5.1, 5.2. A clear temperature gradient was found within the vial as heated or cooled air mixed with ambient air (23°C). Temperature affected gradient; with higher temperatures (27.197 ± 0.894 SD) recorded at the top of the vial in the 36°C testing vial, no gradient found within the 25°C testing vials (24.609 ± 0.423 SD), and lower temperatures (23.697 ± 0.423 SD) recorded at the top of the vial in the 15°C testing vials. Experimental setup (open cage or closed vial) and treatment (resource or no resource) did not make a difference to the temperature gradient recorded.

Appendix B Table 5.1: Mean deviations and an accuracy measure (RMSE) for data collected at the top and bottom of vials for both the closed vial and open cage setups, with vials that have resources or are empty (no resource).

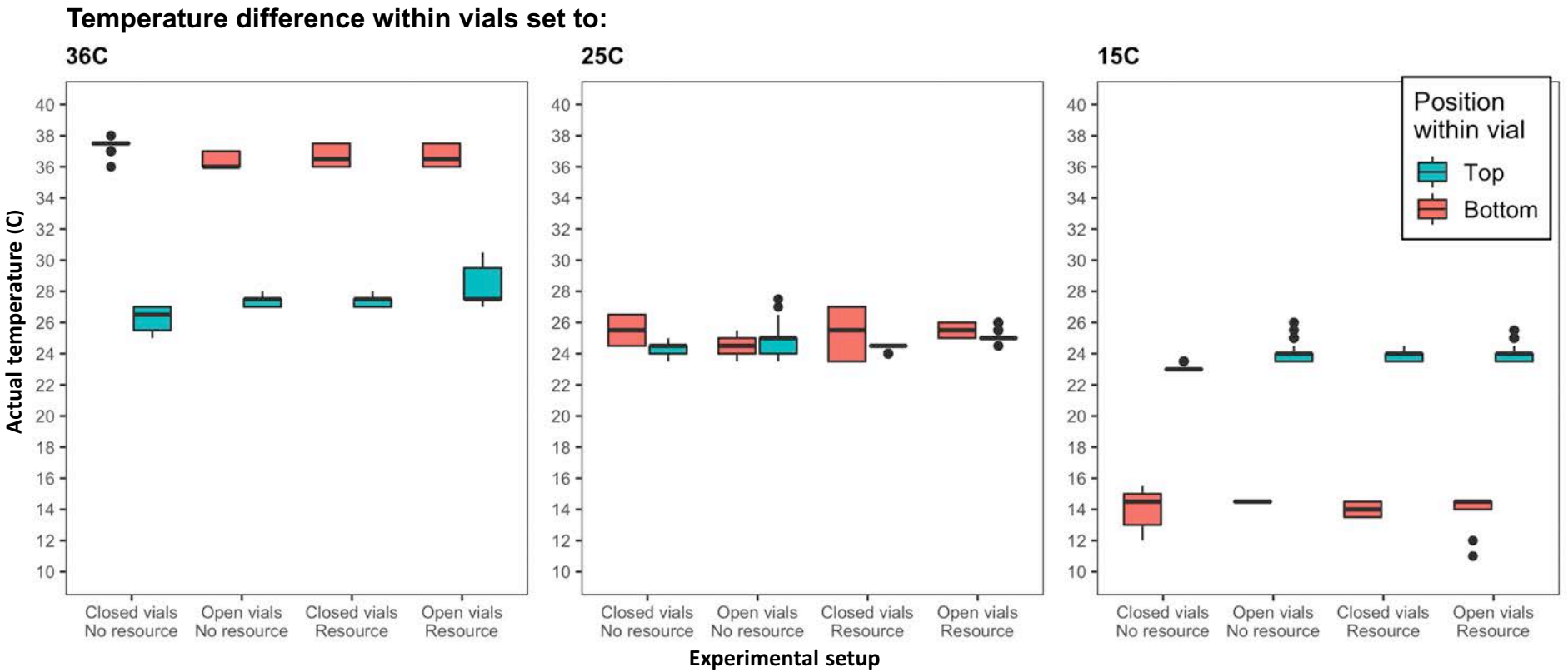
Target Temperature (°C)	**Statistic**	**CLOSED VIAL**				**OPEN CAGE**			
		Bottom[1]		**Top[2]**		**Bottom[1]**		**Top[2]**	
		Resource	**No Resource**	**Resource**	**No Resource**	**Resource**	**No Resource**	**Resource**	**No Resource**
15°C	Mean deviation ± sd	−0.80 ± 0.50	−0.80 ± 0.95	8.78 ± 0.25	8.50 ± 0.21	−0.67°C ± 0.25	−0.50°C ± 0.00	8.89°C ± 0.35	8.86°C ± 0.39
	RMSE	0.50	0.95	0.25	0.21	0.25	1.28 x e-^{13}	0.35	0.39
25°C	Mean deviation ± sd	0.33 ± 1.43	0.39 ± 0.91	−0.54 ± 0.13	−0.67 ± 0.24	0.55 ± 0.43 SD	−0.44 ± 0.58	0.05 ± 0.16 SD	−0.49 ± 0.53 SD
	RMSE	1.43	0.91	0.13	0.24	0.43	0.58	0.16	0.53
36°C	Mean deviation ± sd	0.634 ± 0.63	1.46 ± 0.14	8.62 ± 0.24	9.67 ± 0.63	0.67 ± 0.62 SD	0.49 ± 0.50 SD	7.85 ± 1.08 SD	8.73 ± 0.26 SD
	RMSE	0.63	0.13	0.24	0.63	0.62	0.49	1.08	0.26

Position of data logger in vial	**Experimental design**	**RMSE (C°)**
Bottom	Closed vial	0.92
	Open cage	0.56
Top	Closed vial	0.62
	Open cage	0.74

Notes: [1] indicates data logger was placed at the bottom of the vial either directly on the resource or in the empty vial. [2] indicates the data logger was placed 2 cm from the top of the vial.



Appendix B Figure 5.1: Raw temperature deviations from the validation tests for several niche landscapes over short- and long-term durations.



Appendix B Figure 5.2: Boxplots showing how temperature varies within vials (at the bottom and top of the vial) for three different set temperatures for both experimental setups and with resources present or not present.

Appendix B Supplementary Material 6: The following table shows a list of thermal apparatuses similar to ours and that have, or can be, used for experimental ecology and evolution. This is not a comprehensive list, but includes, to the best of our knowledge, similar equipment. The table outlines how our equipment differs from other works in 5 important aspects (i.e., Ecological and evolutionary potential investigations, temperature settings, operation and setup, replication, and temperature range).

Reference	Description and overall function	Point of difference		
		Equipment facet	**Prior work**	**Our work**
Greenspan, S.E., Morris, W., Warburton, R., Edwards, L., Duffy, R., Pike, D.A., … Alford, R.A. (2016). Low-cost fluctuating-temperature chamber for experimental ecology. Methods in Ecology and Evolution, 7(12), 1567–1574. doi: 10.1111/2041-210X.12619	Describes a low-cost incubator used to create thermal cycles/regimes for experimental ecology.	Ecological and evolutionary potential investigations	- Physiological tolerances and thermal limits, thermoregulation behaviour, plasticity, experimental evolution, incubation of small model organisms	- Physiological tolerances and thermal limits, species interactions, thermoregulation behaviour, plasticity, experimental evolution, incubation of small model organisms
		Temperature settings	- Single temperature - Constant or dynamic temperature regime	- Single temperature or thermal gradient with specified middle temperatures - Constant or dynamic temperature regime
		Operation and setup	- Single, large unit - Controlled via microcontroller connected to Peltier cooler and digital temperature sensor - Low-cost and parts are commercially available	- Series of small, independent units - Controlled via microcontroller connected to Peltier coolers, digital temperature sensors, tri-coloured LED light, and water-cooling system - Robust and parts are commercially available - Fully customizable
		Replication	- Replicates can be within or across incubators	- Replicates can be within or across incubators
		Temperature range	- 13.1°–35.5°C	- 8°– 42°C
Kong, D.J, Axford, J.K., Hoffmann, A.A., & Kearney, M.R. (2016). Novel applications of thermocyclers for phenotyping invertebrate thermal responses. Methods in Ecology and Evolution, 7(10), 1201–1208. doi: 10.1111/2041-210X.12589	Tests thermocyclers as incubators to assess thermal responses in eggs.	Ecological and evolutionary potential investigations	- Thermal tolerance and thermal physiology, incubation of very small model organisms (uses PCR tubes)	- Physiological tolerances and thermal limits, species interactions, thermoregulation behaviour, plasticity, experimental evolution, incubation of small model organisms
		Temperature settings	- Single temperature or thermal gradient within thermocycler - Constant or dynamic temperature regime	- Single temperature or thermal gradient with specified middle temperatures - Constant or dynamic temperature regime
		Operation and setup	- Bought two types of ‘Biometra thermocyclers’ (Gottingen, Germany) - Not “do-it-yourself” and therefore not customizable	- Controlled via microcontroller connected to Peltier coolers, digital temperature sensors, tri-coloured LED light, and water-cooling system - Robust and parts are commercially available.
		Replication	- Replicates nested within thermocycler	- Replicates can be within or across incubators

		Temperature range	- 3°– 99°C	- 8°– 42°C
Rajpurohit, S. & Schmidt, P.S. (2016). Measuring thermal behaviour in smaller insects: A case study in *Drosophila melanogaster* demonstrates effects of sex, geographic origin, and rearing temperature on adult behaviour. Fly, 10(4), 149–161. doi: 10.1080/19336934.2016.1194145		Ecological and evolutionary potential investigations	- Thermal behaviour and preferences	- Physiological tolerances and thermal limits, species interactions, thermoregulation behaviour, plasticity, experimental evolution, incubation of small model organisms
		Temperature settings	- Thermal gradient - Gradient across bar is variable due to the linear fashion of the water thermal gradient - Constant temperature regime	- Single temperature or thermal gradient with specified middle temperatures - Constant or dynamic temperature regime
		Operation and setup	- An aluminium bar with two Peltier coolers on either end of the bar and mounted over heats-sink	- Controlled via microcontroller connected to Peltier coolers, digital temperature sensors, tri-coloured LED light, and water-cooling system - Robust and parts are commercially available
		Replication	- Replicates across bars.	- Replicates can be within or across incubators
		Temperature range	- 12°– 32°C	- 8°– 42°C
Wolfe, G.V., Reeder, W.H., & Ervin, B. (2014). Novel materials enable a low-cost temperature-light gradient incubator for microbial studies. Journal of Microbiological Methods, 97, 29–33. doi: 10.1016/j.mimet.2013.12.001	Describes a low-cost incubator used to study growth of microbial communities, with temperature and LED light controls.	Ecological and evolutionary potential investigations	- Thermal tolerances and thermal physiology, incubation of very small species (designed for algae and bacteria cultures)	- Physiological tolerances and thermal limits, species interactions, thermoregulation behaviour, plasticity, experimental evolution, incubation of small model organisms
		Temperature settings	- Thermal gradient - Gradient across wells is variable due to the linear fashion of the water thermal gradient - Constant temperature regime	- Single temperature or thermal gradient with specified middle temperatures - Constant or dynamic temperature regime
		Operation and setup	- Low-cost, but fragile block material with thermal conductivity similar to aluminium (Kfoam: graphite foam material) - Heating/cooling takes ~1hr to reach target temperatures and form gradient	- Controlled via microcontroller connected to Peltier coolers, digital temperature sensors, tri-coloured LED light, and water-cooling system - Robust and parts are commercially available - Heating/cooling takes ~5mins to reach target temperatures - Fully customizable
		Replication	- Replicates nested within incubator.	- Replicates can be within or across incubators
		Temperature range	- 12°–48°C	- 8°– 42°C

Woods, A.H. & Bonnecaze, R.T. (2006). Insect eggs at a transition between diffusion and reaction limitation: temperature, oxygen, and water. Journal of Theoretic Biology, 243(4), 483–492. doi: 10.1016/j.jtbi.2006.07.008	Describes a thermal gradient bar for testing development time of eggs along a thermal gradient.	Ecological and evolutionary potential investigations	- Thermal tolerance and thermal physiology, incubation of very small model organisms (uses PCR tubes)	- Physiological tolerances and thermal limits, species interactions, thermoregulation behaviour, plasticity, experimental evolution, incubation of small model organisms
		Temperature settings	- Thermal gradient - Gradient across wells is variable due to the linear fashion of the thermal gradient - Constant temperature regime	- Single temperature or thermal gradient with specified middle temperatures - Constant or dynamic temperature regime
		Operation and setup	- Temperature controlled via constantly circulating temperature baths.	- Controlled via microcontroller connected to Peltier coolers, digital temperature sensors, tri-coloured LED light, and water-cooling system - Robust and parts are commercially available
		Replication	- Replicates nested within thermal gradient bar.	- Replicates can be within or across incubators
		Temperature range	- 22°– 32°C	- 8°– 42°C
Elsgaard, L. & Jorgensen, L.W. (2002). A sandwich-designed temperature-gradient incubator for studies of microbial temperature responses. Journal of Microbiological Methods, 49(1): 19–29. doi: 10.1016/S0167-7012(01)00361-X	Describes a thermal gradient used to study microbial growth.	Ecological and evolutionary potential investigations	- Thermal tolerances and thermal physiology, incubation of very small species (designed for algae and bacteria cultures)	- Physiological tolerances and thermal limits, species interactions, thermoregulation behaviour, plasticity, experimental evolution, incubation of small model organisms
		Temperature settings	- Thermal gradient - Gradient across wells is variable due to the linear fashion of the thermal gradient - Constant temperature regime	- Single temperature or thermal gradient with specified middle temperatures - Constant or dynamic temperature regime
		Operation and setup	- Temperature controlled at hot end using an electric plate and at cold end using a Peltier cooler and liquid cooling system	- Controlled via microcontroller connected to Peltier coolers, digital temperature sensors, tri-coloured LED light, and water-cooling system - Robust and parts are commercially available - Fully customizable
		Replication	- Replicates nested within incubator	- Replicates can be within or across incubators
		Temperature range	- 0°–40°C with high precision (±0.08°C)	- 8°– 42°C with precision of ±0.5°C

Thomas, T.H., Scotten, H.L., Bradshow, J.S. (1963). Thermal gradient incubators for small aquatic organisms." Limnology and Oceanography, 357-360. doi: 10.4319/lo.1963.8.3.0357	Describes three thermal gradient "blocks" used to incubate algae, bacteria, and foraminifera cultures.	Ecological and evolutionary potential investigations	- Thermal tolerances and thermal physiology, incubation of very small species (designed for algae and bacteria cultures)	- Physiological tolerances and thermal limits, species interactions, thermoregulation behaviour, plasticity, experimental evolution, incubation of small model organisms
		Temperature settings	- Thermal gradient - Gradient across wells is variable due to the linear fashion of the water thermal gradient - Constant temperature regime - Heating/cooling takes ~1hr to reach target temperatures and form gradient.	- Single temperature or thermal gradient with specified middle temperatures - Constant or dynamic temperature regime Heating/cooling takes ~5mins to reach target temperatures.
		Operation and setup	- Thermal gradient is created by circulating cold water at one end and hot water at opposite end of an aluminium block, and vials are placed in holes drilled into block. - Requires shaking of module continuously to achieve an approximately linear temperature gradient across module, which could disrupt living organisms	- Controlled via microcontroller connected to Peltier coolers, digital temperature sensors, tri-coloured LED light, and water-cooling system - Fully customizable
		Replication	- Replicates nested within one "block"	- Replicates can be within or across incubators
		Temperature range	- 10°–30°C	- 8°– 42°C
Lu, H., Land, B., & Johnson, B. Peltier Temperature Controller. Cornell University ME Design Project Report. Cornell University, New York.	Describes a low-cost controller used to control a bath temperature for petri dishes used in Drosophila experiments.	Ecological and evolutionary potential investigations	- Thermal physiology	- Thermal physiology, thermoregulation behaviour, species interactions, plasticity, experimental evolution, incubation of small model organisms
		Temperature settings	- Creates thermal pulses to a petri dish. - Single temperature - Constant temperature regime	- Single temperature or thermal gradient with specified middle temperatures - Constant or dynamic temperature regime
		Operation and setup	- A feedback control box and temperature box are used to control and regulate a Peltier cooler to heat/cool wells - Each are separate units and able to connect to each other via plugs	- Controlled via microcontroller connected to Peltier coolers, digital temperature sensors, tri-coloured LED light, and water-cooling system - Fully customizable
		Replication	- Replicates nested within or across thermal baths	- Replicates can be within or across incubators
		Temperature range	- 15°–35°C	- 8°– 42°C

Appendix C: Supporting information for Chapter 4

Appendix C Table 1: Pre-defined functions used to fit non-linear least square models.

Functions were fit to each population and species dataset (*N* = 4 per temperature point) to determine the function that best represented each individual dataset for parametrization of thermal performance curves.

Model name	Equation	Reference
Gaussian	$Performance = P_{max} \times exp^{\left(-0.5\left(\frac{\lvert temp - T_{opt}\rvert}{a}\right)^2\right)}$	Lynch, M., Gabriel, W. (1987). Environmental tolerance. The American Naturalist. 129, 283–303.
Type 1 modified Gaussian 2006	$Performance = P_{max} \times exp^{\left(-0.5\left(\frac{\lvert temp - T_{opt}\rvert}{a}\right)^b\right)}$	Angilletta Jr, M. J. (2006). Estimating and comparing thermal performance curves. Journal of Thermal Biology, 31(7), 541-545.
Type 2 modified Gaussian 2008	$Performance = \begin{cases} P_{max} \times exp^{\left(-\left(\frac{\lvert temp - T_{opt}\rvert}{2 \times c^2}\right)^2\right)}, & x < T_{opt} \\ P_{max} \times exp^{\left(-\left(\frac{\lvert temp - T_{opt}\rvert}{2 \times (c \times d)^2}\right)^2\right)}, & x \geq T_{opt} \end{cases}$	Phillips et al. (2014). Do evolutionary constraints on thermal performance manifest at different organizational scales? Journal of Evolutionary Biology. 27, 2687-2694.
beta	$Performance = \frac{a\left(\frac{temp - b + \frac{c(d-1)}{d+e-2}}{c}\right)^{d-1} \times \left(1 - \frac{temp - b + \frac{c(d-1)}{d+e-2}}{c}\right)^{e-1}}{\left(\frac{d-1}{d+e-2}\right)^{d-1} \times \left(\frac{e-1}{d+e-2}\right)^{e-1}}$	Niehaus, Amanda C., et al. (2012). Predicting the physiological performance of ectotherms in fluctuating thermal environments. Journal of Experimental Biology 215.4: 694-701.
quadratic	$Performance = a + b + temp + c + temp^2$	Montagnes, David JS, et al. (2008). Short-term temperature change may impact freshwater carbon flux: a microbial perspective. Global Change Biology 14.12: 2823-2838.
cubic	$Performance = a + b + temp + c + temp^2 + d + temp^3$	
quartic	$Performance = a + b + temp + c + temp^2 + d + \ temp^4 + e + temp^4$	

Appendix C Table 2: Productivity non-linear least square model fit scores.

Models with the lowest AICc score were chosen as the best fit for parametrization of productivity thermal performance curves. Models with a AICc score < 2 from the lowest AICc value (see ∆AICc) were included in model averaging for parametrization. Models weighted by the inverse of the variance.

Species	Population	Model name	sigma	∆AICc	AIC	AICc	BIC	df.residual
D. serrata	Granite	beta	3.78	72	2956	2965	2961	12
		Type2.modgaussian.2008	1.06	0	2887	2893	2891	13
		gaussian.1987	4.44	75	2964	2968	2968	14
		Type1.modgaussian.2006	1.32	11	2898	2903	2902	13
	Raglan	beta	4.7	65	2937	2945	2942	12
		Type2.modgaussian.2008	2.14	17	2892	2897	2896	13
		gaussian.1987	4.33	61	2937	2941	2941	14
		Type1.modgaussian.2006	1.31	0	2874	2880	2878	13
D. birchii	Mt Lewis	beta	1.85	6	5139	5147	5144	12
		Type2.modgaussian.2008	1.88	2	5138	5143	5142	13
		gaussian.1987	1.91	0	5138	5141	5141	14
		Type1.modgaussian.2006	1.91	2	5137	5143	5142	13
	Paluma	beta	1.46	19	4522	4531	4527	12
		Type2.modgaussian.2008	1.47	15	4522	4527	4526	13
		gaussian.1987	1.51	31	4539	4543	4543	14
		Type1.modgaussian.2006	1.15	0	4507	4512	4511	13

Appendix C Table 3: Development speed non-linear least square model fit scores.

Models with the lowest AICc score were chosen as the best fit for parametrization of development speed thermal performance curves. Models with a AICc score < 2 from the lowest AICc value (see ∆AICc) were included in model averaging for parametrization. Models were not weighted.

Species	Population	Model name	sigma	∆AICc	AIC	AICc	BIC	df.residual
D. serrata	Granite	gaussian.1987	0.00525	0.5	-95	-90	-92.8	10
		Type2.modgaussian.2008	0.00438	0	-99.1	-90.5	-96.2	9
		Type1.modgaussian.2006	0.00513	4.1	-95	-86.4	-92.1	9
		beta	0.00487	8.7	-95.8	-81.8	-92.4	8
	Raglan	gaussian.1987	0.00502	0	-96.2	-91.2	-93.9	10
		modgaussian.2008	0.00487	3.4	-96.3	-87.8	-93.5	9
		modgaussian.2006	0.00494	3.8	-95.9	-87.4	-93.1	9
		beta	0.00531	11.6	-93.6	-79.6	-90.2	8
D. birchii	Mt Lewis	gaussian.1987	0.00931	33.2	-67.2	-60.5	-65.6	8
		Type2.modgaussian.2008	0.00244	9.6	-96.1	-84.1	-94.2	7
		Type1.modgaussian.2006	0.00817	36.2	-69.5	-57.5	-67.5	7
		beta	0.00103	0	-115	-93.7	-112	6
	Paluma	gaussian.1987	0.00445	1.7	-91.4	-85.6	-89.4	9
		Type2.modgaussian.2008	0.00448	6.7	-90.6	-80.6	-88.2	8
		Type1.modgaussian.2006	0.0034	0	-97.3	-87.3	-94.8	8
		beta	0.00481	15.6	-88.5	-71.7	-85.6	7

Appendix C Table 4: Wing size non-linear least square model fit scores.

Models with the lowest AICc score were chosen as the best fit for parametrization of wing size thermal performance curves. Models with a AICc score < 2 from the lowest AICc value (see ∆AICc) were included in model averaging for parametrization. Models were not weighted.

Species	Population	Model name	sigma	∆AICc	AIC	AICc	BIC	df.residual
D. serrata	Granite	gaussian.1987	6.32	0	82.9	88.6	84.8	9
		Type1.modgaussian.2006	6.13	4.1	82.7	92.7	85.1	8
		beta	6.45	12.4	84.3	101	87.3	7
		quartic	6.45	12.4	84.3	101	87.2	7
		cubic	6.26	4.6	83.2	93.2	85.6	8
		quadratic	6.4	0.3	83.2	88.9	85.1	9
	Raglan	gaussian.1987	6.69	0.1	90.9	95.9	93.2	10
		Type1.modgaussian.2006	6.97	5.2	92.6	101	95.4	9
		beta	7.42	13.2	94.7	109	98.1	8
		quartic	6.7	10.2	92	106	95.4	8
		cubic	6.99	5.2	92.7	101	95.5	9
D. birchii	Mt Lewis	quadratic	6.66	0	90.8	95.8	93	10
		gaussian.1987	13	0.5	92.1	98.8	93.7	8
		Type1.modgaussian.2006	11.7	3.7	90.4	102	92.4	7
		beta	12.5	14.7	92.1	113	94.5	6
		quartic	11.7	13.7	90.6	112	93	6
		cubic	12.9	6.7	92.6	105	94.6	7
		quadratic	12.7	0	91.7	98.3	93.3	8
	Paluma	gaussian.1987	15.4	11.5	95.9	103	97.5	8
		Type1.modgaussian.2006	12.2	11.5	91.2	103	93.2	7
		beta	14.7	25.5	95.7	117	98	6
		quartic	7.44	10.5	80.7	102	83.1	6
		cubic	7.14	0	79.5	91.5	81.5	7
		quadratic	15.6	11.5	96.2	103	97.8	8

Appendix C Table 5: Temperature preference non-linear least square model fit scores.

Models with the lowest AICc score were chosen as the best fit to determine temperature preference. Models with a AICc score < 2 from the lowest AICc value (see ∆AICc) were included in model averaging for parametrization. Models weighted by the inverse of the variance.

Species	Population	Model name	sigma	∆AICc	AIC	AICc	BIC	df.residual
D. serrata	Granite	beta	2.91	9	4106	4114	4111	12
		modgaussian.2008	2.43	0	4098	4103	4102	13
		gaussian.1987	2.68	3	4101	4105	4105	14
		modgaussian.2006	2.86	6	4104	4109	4108	13
	Raglan	beta	3.12	7	4216	4224	4221	12
		modgaussian.2008	3.12	4	4215	4221	4219	13
		gaussian.1987	2.96	6	4219	4223	4223	14
		modgaussian.2006	2.92	0	4212	4217	4216	13
D. birchii	Mt Lewis	beta	9.14	0	128	132	132	14
		modgaussian.2008	8.97	2	128	134	132	13
		gaussian.1987	8.49	0	126	132	131	13
		modgaussian.2006	9.64	6	131	140	136	12
	Paluma	beta	1.39	9	6175	6184	6180	12
		modgaussian.2008	1.28	0	6169	6175	6173	13
		gaussian.1987	1.34	3	6175	6178	6178	14
		modgaussian.2006	1.32	0	6170	6175	6174	13

Appendix C Table 6: Thermal performance curve parameters.

TPC parameters and confidence intervals are shown in grey.

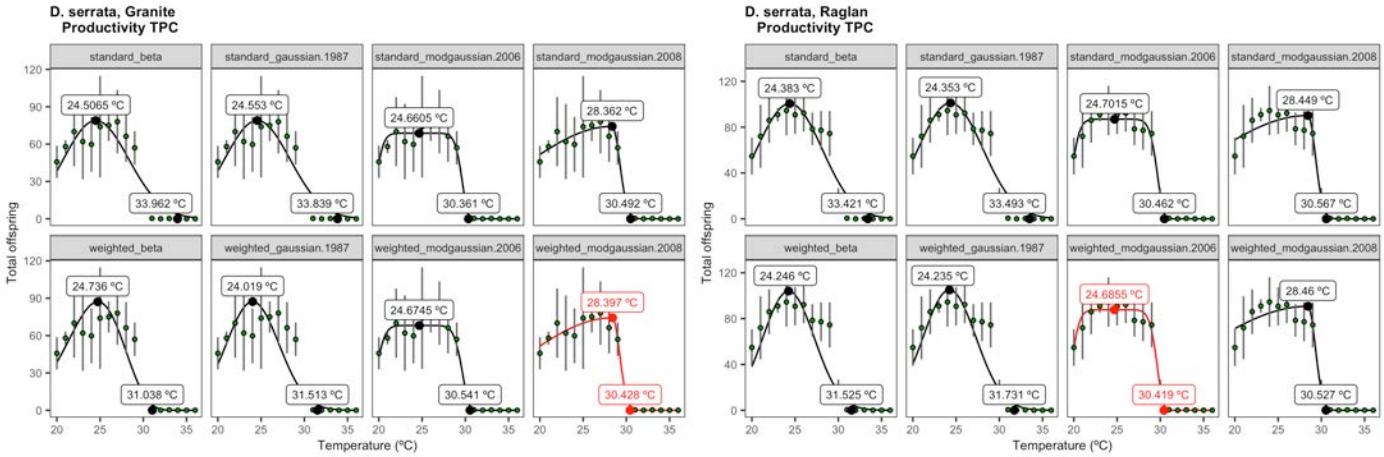
Trait	Species	Population	T_{opt} (C°)	T_{opt} CI (C°)	P_{max}	P_{max} CI	B_{80} (C°)
Productivity	*D. serrata*	Granite	28.40	(28.09, 28.71)	74.28	(68.99, 79.56)	7.14
		Raglan	24.69	(24.52, 24.85)	87.72	(83.68, 91.76)	8.56
	D. birchii	Mt Lewis	23.61	(23.21, 24.01)	64.35	(55.38, 73.32)	3.44
		Paluma	23.64	(23.43, 23.86)	60.06	(54.59, 65.53)	4.44
Development speed	*D. serrata*	Granite	27.29	(26.14, 27.45)	0.111	(0.106, 0.116)	10.06
		Raglan	26.64	(26.06, 27.21)	0.110	(0.106, 0.115)	10.46
	D. birchii	Mt Lewis	28.74	(26.14, 28.95)	0.126	(0.107, 0.1268	6.49
		Paluma	26.75	(26.07, 27.11)	0.113	(0.108, 0.117)	9.79
Wing size	*D. serrata*	Granite	20.00	(7.71, 22.81)	980.69	(952.92, 1033.91)	11.00
		Raglan	20.00	(12.48, 21.71)	974.23	(955.96, 1003.01)	13.00
	D. birchii	Mt Lewis	20.00	(16.03, 23.60)	930.12	(905.45, 954.91)	10.00
		Paluma	21.55	(16.34, 24.01)	942.41	(906.1, 960.81)	13.00

Appendix C Table 7: Temperature preference for *D. serrata* and *D. birchii*.

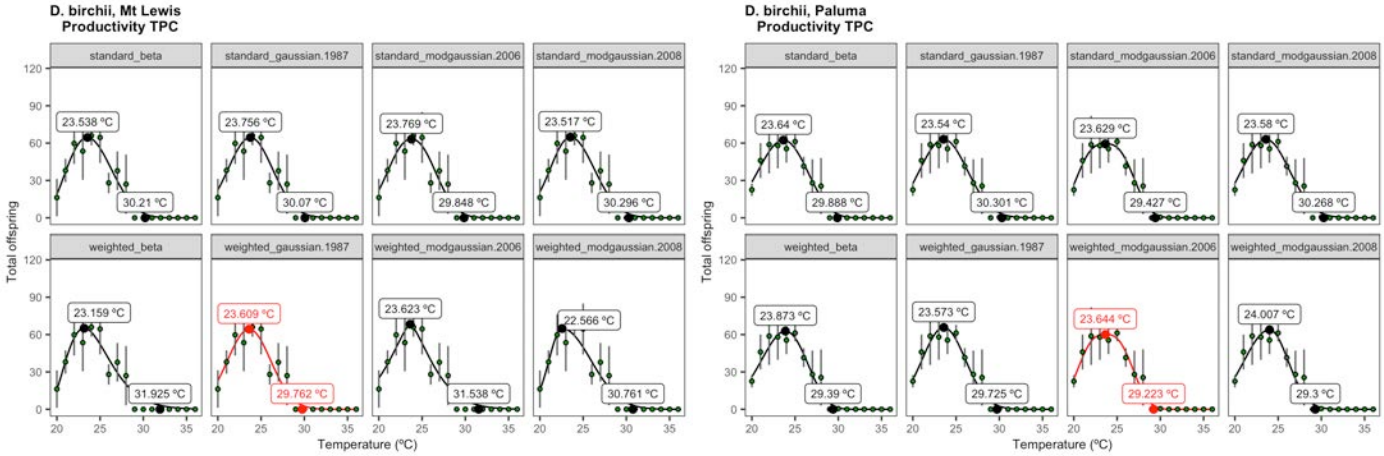
T_{pref}, temperature preference percentiles T_{set}, and peak temperature preference range (B_{80}) for each population and species. Confidence intervals are also shown.

Species	Population	T_{pref} (C°)	T_{pref} CI (C°)	80% T_{set} (C°)	80% T_{set} CI (C°)	B_{80}
D. serrata	Granite	28.40	(27.057, 29.734)	25.49 – 28.89	(24.51, 26.63) – (28.68, 29.01)	2.943
	Raglan	25.43	(24.642, 26.219)	23.78 – 27.11	(23.16, 24.36) – (26.62, 27.69)	7.436
D. birchii	Mt Lewis	24.07	(20, 28.678)	21.95 – 25.33	(21.38, 22.62) – (24.72, 25.89)	4.719
	Paluma	25.35	(24.556, 26.152)	24.24 – 27.59	(23.46, 24.87) – (26.95, 28.20)	2.581

D. serrata (generalist)

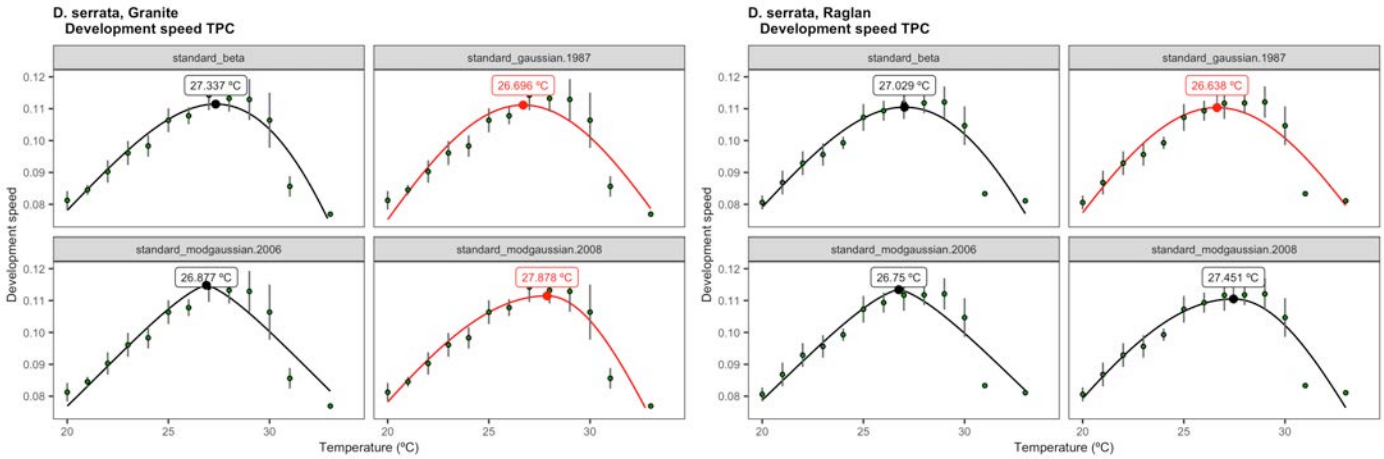


D. birchii (specialist)

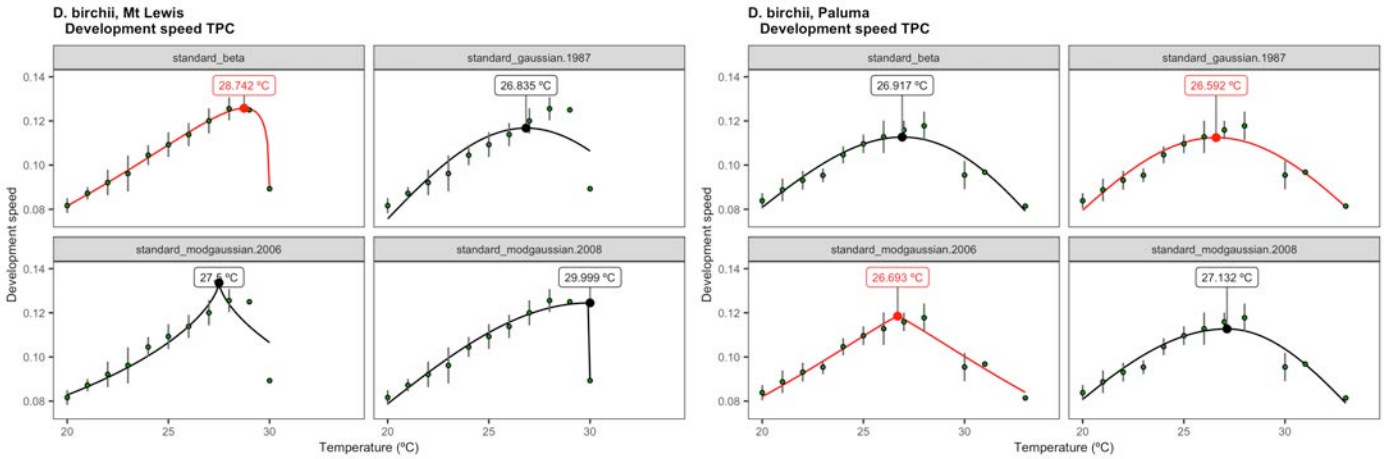


Appendix C Figure 1. Standard and weighted model fit to four pre-defined functions for productivity of a generalist and specialist species.

D. serrata (generalist)

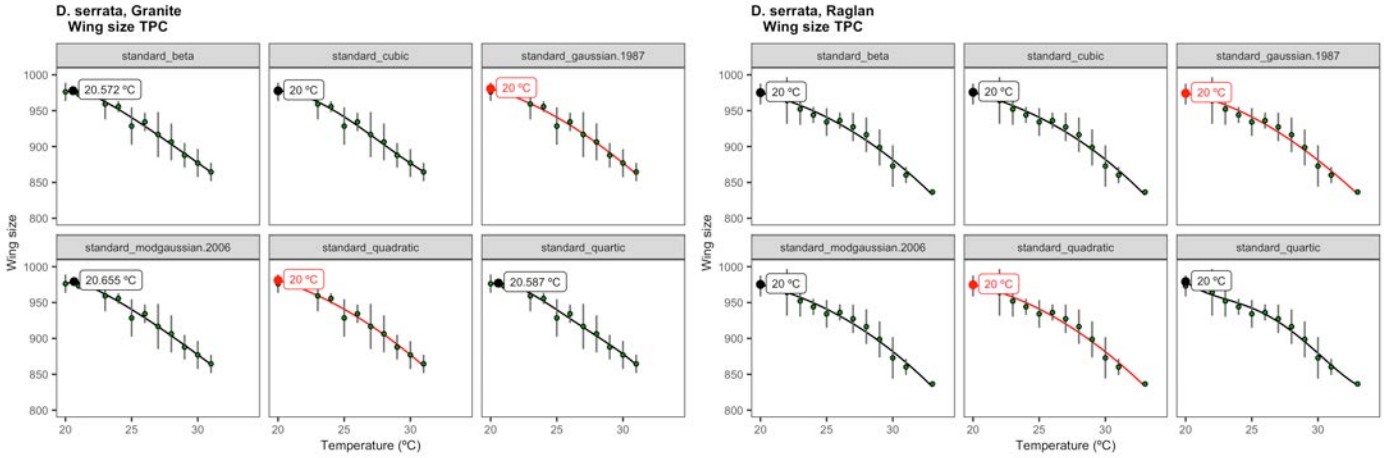


D. birchii (specialist)

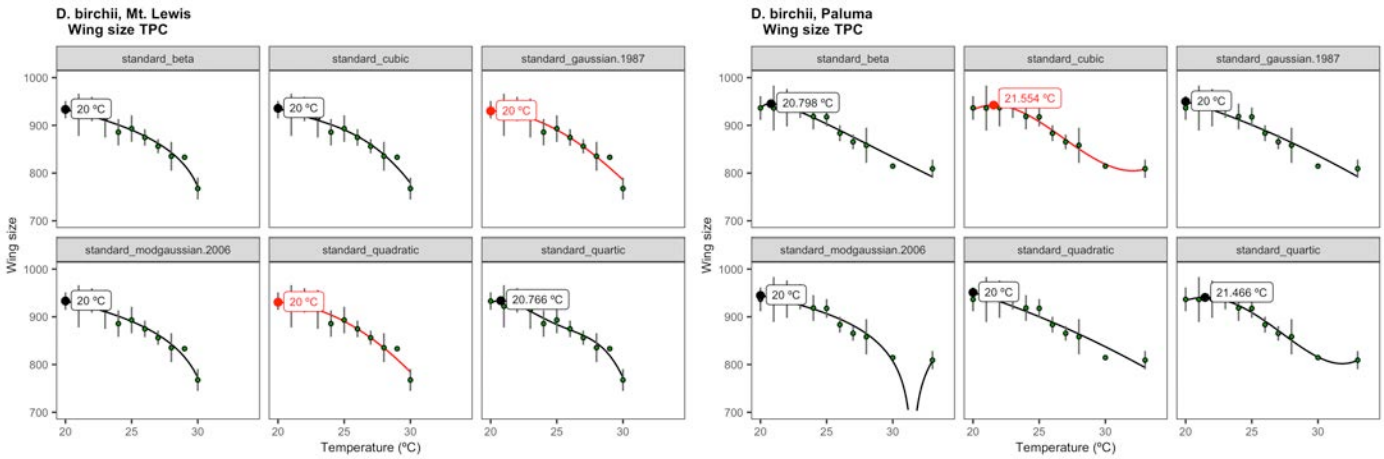


Appendix C Figure 2. Standard model fit to four pre-defined functions for development speed of a generalist and specialist species.

D. serrata (generalist)

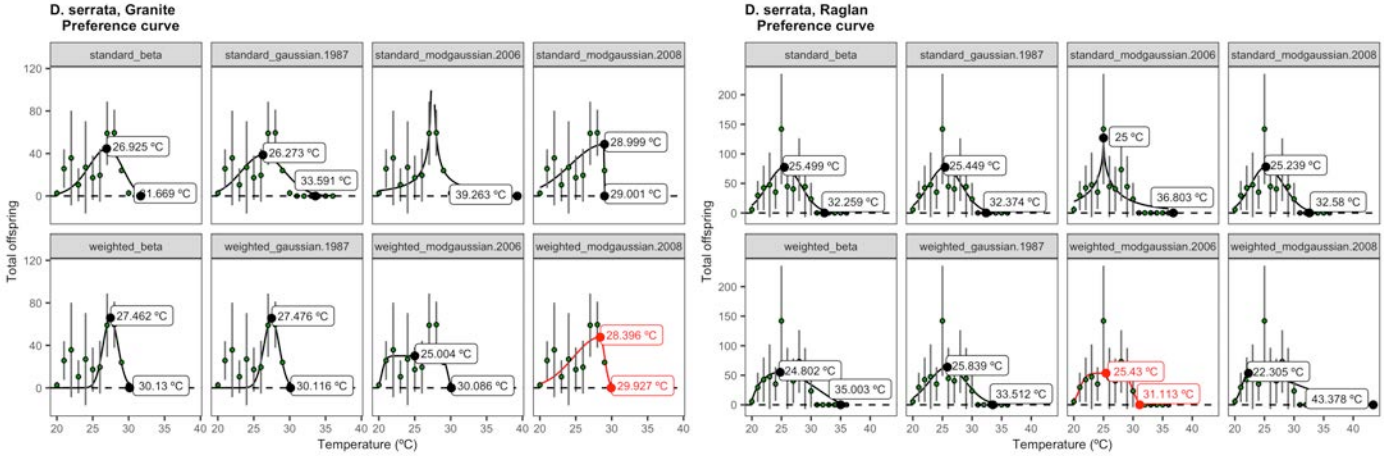


D. birchii (specialist)

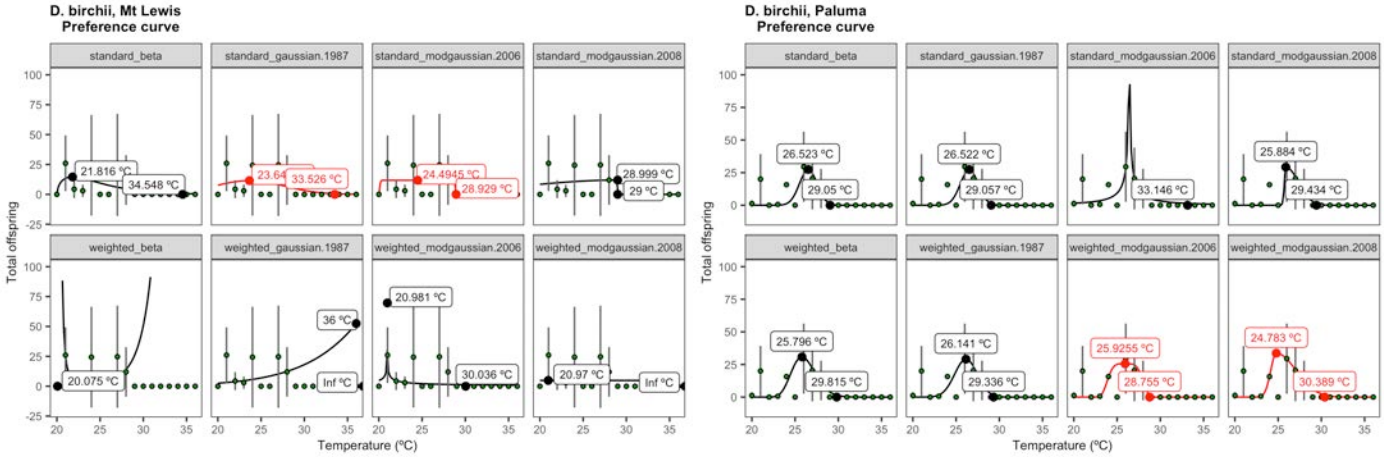


Appendix C Figure 3. Standard model fit to four pre-defined functions for wing size of a generalist and specialist species.

D. serrata (generalist)



D. birchii (specialist)



Appendix C Figure 4. Standard and weighted model fit to four pre-defined functions for temperature preference of a generalist and specialist species.

Appendix D: Supporting information for Chapter 5

Appendix D Table 1: **Heritability analysis.**

A. Broad-sense heritability (H^2) for static heat knockdown time (KD_T) in *Drosophila birchii* populations obtained from a variance component analysis in isofemale lines. Estimates include additive variance (V_A), between-line variance ($V_{between}$), within-line (V_{within}), and total variance (V_{total}). **B**. Variance components with lower and upper confidence intervals (CI) and ANOVA *P*-values obtained by comparing REML models with and without the factor of interest.

A. Heritability estimates

Population	H^2	*95% CI* for H^2	V_A	$V_{between}$	V_{within}	V_{total}
Mt Lewis	0.60	0.53, 0.73	3.38	2.00	3.62	5.62
Paluma	0.49	0.14, 0.73	3.31	1.96	4.74	6.70

B. Variance component estimates

Population	Factor	*Variance*	*P*-value	Lower *CI* (2.5%)	Upper *CI* (97.5%)
Mt Lewis	Run	1.14	*0.002*	0.60	1.61
	Line	2.00	0.04	0.44	1.93
	Replicate	2.55	*0.07*	0.00	2.15
	Residual	1.07	-	0.58	2.08
Paluma	Run	0.62	*< 0.001*	0.00	1.31
	Line	1.96	0.04	0.17	1.98
	Replicate	-	-	-	-
	Residual	4.74	-	1.83	2.62

Appendix D Table 2: **The expected response to selection (i.e., change in trait means) for heat knockdown time after the intial heatwave ('heatwave 1') and the GLMM estimates and effects for heat knockdown time in the second heatwave ('heatwave 2').**

A. Expected changes in trait means calculated from the following equation: $R_f = i\sigma_f h_f^2$, where the intensity of selection (i), the observed standard deviation of family means (σ_f), and the heritability of family means (h_f^2) are used to calculate the expected response (R_f). **B**. Estimated regression parameters, standard errors, *t*-values, *P*-values and the R^2 value for the gamma GLMM fit to knockdown time data for the second static heat knockdown assay ('heatwave 2'). **C.** Random variance component estimates and the intraclass correlation for random structure of gamma GLMM.

A. Expected response to selection

Population	Treatment	i	σ_f	h^2	R_f	ΔKD_t
Paluma	Control	0.00	2.69	0.49	0.000	15.69
	Moderate	0.79	2.52	0.49	0.977	16.67
	Severe	1.73	3.07	0.49	2.607	18.30
Mt Lewis	Control	0.00	2.58	0.60	0.000	16.13
	Moderate	0.79	2.46	0.60	1.170	17.30
	Severe	1.73	2.14	0.60	2.222	18.35

B. Treatment effects

	Estimate ± *SE**	*t* value	*P*-value	Lower *CI* (2.5%)*	Upper *CI* (97.5%)*	R^2
Intercept	19.910 ±1.030	100.21	*<0.001*	18.72763	21.11534	0.995
Moderate	-0.141 ±1.014	-0.494	0.621	-0.64774	0.44812	
Severe	0.404 ±1.015	1.396	0.163	-0.15164	1.04712	

C. Variance component estimates

Factor	*Variance*	*SD*	Intraclass correlation
Mass bred	0.0012	0.0347	0.994
Run	0.0014	0.0373	
Vial	0.0007	0.0262	
Residual	0.0075	0.0866	

*The asterisk indicates the estimate was back-transformed from the log scale because the gamma GLMM used the 'log' link.

Appendix D Table 3. Productivity non-linear least square model fit scores.

Models were weighted by the inverse of the variance. The model with the lowest AICc score was chosen as the best fit for parametrization of productivity thermal performance curves. Models noted with an asterisk were the model with the second lowest AICc score and used for parameterization due to extreme parameter predictions and visual misfit of the data from the model with the lowest AICc score.

Treatment	Population	Model name	sigma	∆AICc	AIC	AICc	BIC	df.residual
Control	Mt Lewis	modgaussian.2008	0.505	6	5460	5465	5464	13
		gaussian.1987	0.506	0	5456	5459	5459	14
		modgaussian.2006	0.502	3	5456	5462	5460	13
	Paluma	modgaussian.2008	0.456	9	4811	4817	4815	13
		gaussian.1987	0.564	79	4884	4887	4887	14
		modgaussian.2006	0.327	0	4803	4808	4807	13
Moderate	Mt Lewis	modgaussian.2008	0.211	2	4848	4853	4852	13
		gaussian.1987	1.17	95	4943	4946	4946	14
		modgaussian.2006*	0.171	0	4846	4851	4850	13
	Paluma	modgaussian.2008	0.424	0	5416	5422	5420	13
		gaussian.1987	0.678	23	5442	5445	5445	14
		modgaussian.2006	0.448	0	5417	5422	5421	13
Severe	Mt Lewis	modgaussian.2008	0.739	38	5888	5894	5892	13
		gaussian.1987*	0.602	35	5888	5891	5891	14
		modgaussian.2006	0.329	0	5850	5856	5854	13
	Paluma	modgaussian.2008	0.289	0	5327	5332	5331	13
		gaussian.1987	0.393	14	5342	5346	5346	14
		modgaussian.2006	0.345	4	5330	5336	5334	13

Appendix D Table 4. Development speed non-linear least square model fit scores.

The model with the lowest AICc score was chosen as the best fit for parametrization of development speed thermal performance curves. Models with a AICc score < 2 from the lowest AICc value (see ∆AICc) were included in model averaging for parametrization. Models were not weighted.

Treatment	Population	Model name	sigma	∆AICc	AIC	AICc	BIC	df.residual
Control	Mt Lewis	gaussian.1987	0.00154	3.8	-96.7	-88.7	-95.5	7
		modgaussian.2008	0.0194	61.9	-45.6	-30.6	-44.1	6
		modgaussian.2006	0.000878	0	-107	-92.5	-106	6
		quadratic	0.00169	5.7	-94.8	-86.8	-93.6	7
		cubic	0.00128	7.5	-100	-85	-98.5	6
		quartic	0.00118	19.1	-101	-73.4	-99.6	5
	Paluma	gaussian.1987	0.0013	0	-89.7	-79.7	-89	6
		modgaussian.2008	0.0157	55.2	-44.5	-24.5	-43.5	5
		modgaussian.2006	0.00121	9.1	-90.6	-70.6	-89.6	5
		quadratic	0.00144	1.8	-87.9	-77.9	-87.1	6
		cubic	0.001	5.6	-94.1	-74.1	-93.1	5
		quartic	0.00111	29.5	-92.2	-50.2	-91	4
Moderate	Mt Lewis	gaussian.1987	0.00226	1.1	-89.1	-81.1	-87.9	7
		modgaussian.2008	0.00147	0	-97.2	-82.2	-95.7	6
		modgaussian.2006	0.0018	3	-93.1	-78.1	-91.6	6
		quadratic	0.00243	2.6	-87.6	-79.6	-86.3	7
		cubic	0.00214	7.6	-89.6	-74.6	-88.1	6
		quartic	0.0012	9.2	-101	-73	-99.2	5
	Paluma	gaussian.1987	0.00147	1.9	-87.5	-77.5	-86.7	6
		modgaussian.2008	0.0181	57.5	-41.9	-21.9	-41	5
		modgaussian.2006	0.00131	10.2	-89.2	-69.2	-88.2	5
		quadratic	0.00132	0	-89.4	-79.4	-88.6	6
		cubic	0.0013	10.1	-89.3	-69.3	-88.4	5
		quartic	0.00145	34	-87.4	-45.4	-86.2	4
Severe	Mt Lewis	gaussian.1987	0.00217	136.3	-52.7	-12.7	-53.5	3
		modgaussian.2008	0.0166	-	-28.8	Inf	-29.8	2
		modgaussian.2006	0.00146	-	-57.9	Inf	-58.9	2
		quadratic	0.00178	133.9	-55.1	-15.1	-56	3
		cubic	0.00116	-	-60.7	Inf	-61.7	2
		quartic	0.000982	0	-64.8	-149	-66.1	1
	Paluma	gaussian.1987	0.00366	0	-71.1	-61.1	-70.3	6
		modgaussian.2008	0.00389	11.5	-69.6	-49.6	-68.7	5
		modgaussian.2006	0.00355	9.8	-71.3	-51.3	-70.3	5
		quadratic	0.00389	1.1	-70	-60	-69.2	6
		cubic	0.00361	10.1	-71	-51	-70	5
		quartic	0.00403	34.1	-69	-27	-67.8	4

Appendix D Table 5. Wing size non-linear least square model fit scores.

The model with the lowest AICc score was chosen as the best fit for parametrization of wing size thermal performance curves. Models with a AICc score < 2 from the lowest AICc value (see ∆AICc) were included in model averaging for parametrization. Models were not weighted.

Treatment	Population	Model name	sigma	∆AICc	AIC	AICc	BIC	df.residual
Control	Mt Lewis	gaussian.1987	9.37	0	77.6	85.6	78.8	7
		modgaussian.2008	44.2	38.4	109	124	111	6
		modgaussian.2006	9.78	8.3	78.9	93.9	80.4	6
		quadratic	9.45	0.1	77.7	85.7	79	7
		cubic	9.34	7.4	78	93	79.5	6
		quartic	10.2	22.4	79.9	108	81.7	5
	Paluma	gaussian.1987	8.2	0	67.8	77.8	68.6	6
		modgaussian.2008	8.98	12	69.8	89.8	70.7	5
		modgaussian.2006	8.61	11.2	69	89	70	5
		quadratic	8.4	0.4	68.2	78.2	69	6
		cubic	8.04	10	67.8	87.8	68.8	5
		quartic	6.72	29.2	64.5	107	65.7	4
Moderate	Mt Lewis	gaussian.1987	12.2	0.6	82.8	90.8	84	7
		modgaussian.2008	13.1	9.5	84.7	99.7	86.2	6
		modgaussian.2006	8.14	0	75.2	90.2	76.7	6
		quadratic	11.9	0.1	82.3	90.3	83.5	7
		cubic	9.61	3.2	78.5	93.5	80	6
		quartic	9.18	15.7	77.8	106	79.6	5
	Paluma	gaussian.1987	12	0	74.6	84.6	75.4	6
		modgaussian.2008	11.4	9.5	74.1	94.1	75.1	5
		modgaussian.2006	12.1	10.6	75.2	95.2	76.2	5
		quadratic	12	0	74.6	84.6	75.4	6
		cubic	12.9	11.7	76.3	96.3	77.3	5
		quartic	11.4	31.4	74	116	75.2	4
Severe	Mt Lewis	gaussian.1987	4.01	120.1	37.5	77.5	36.7	3
		modgaussian.2008	4.83	-	39.3	Inf	38.3	2
		modgaussian.2006	4.91	-	39.5	Inf	38.5	2
		quadratic	4	120.1	37.5	77.5	36.7	3
		cubic	4.88	-	39.5	Inf	38.4	2
		quartic	6.87	0	41.4	-42.6	40.1	1
	Paluma	gaussian.1987	15	0.5	78.6	88.6	79.4	6
		modgaussian.2008	16	11.9	80.2	100	81.2	5
		modgaussian.2006	14.5	10.2	78.3	98.3	79.3	5
		quadratic	14.6	0	78.1	88.1	78.9	6
		cubic	15.1	11	79.1	99.1	80.1	5
		quartic	13.7	30.9	77.4	119	78.6	4

Appendix D Table 6: Pre-defined functions used to fit non-linear least square models.

Functions were fit to each population and treatment data set (*N* = 4 per temperature point) to determine the function that best represented each individual dataset for parametrization of thermal performance curves.

Model name	Equation	Reference
Gaussian	$Performance = P_{max} \times exp\left(-0.5\left(\frac{\lvert temp - T_{opt}\rvert}{a}\right)^2\right)$	Lynch M and Gabriel W. 1987. Environmental tolerance. The American Naturalist. 129, 283–303.
Type 1 modified Gaussian 2006	$Performance = P_{max} \times exp\left(-0.5\left(\frac{\lvert temp - T_{opt}\rvert}{a}\right)^b\right)$	Angilletta MJ. 2006. Estimating and comparing thermal performance curves. Journal of Thermal Biology, 31(7), 541-545.
Type 2 modified Gaussian 2008	$Performance = \begin{cases} P_{max} \times exp\left(-\left(\frac{\lvert temp - T_{opt}\rvert}{2 \times c^2}\right)^2\right), & x < T_{opt} \\ P_{max} \times exp\left(-\left(\frac{\lvert temp - T_{opt}\rvert}{2 \times (c \times d)^2}\right)^2\right), & x \geq T_{opt} \end{cases}$	Phillips et al. 2014. Do evolutionary constraints on thermal performance manifest at different organization scales? Journal of Evolutionary Biology, 27; 2687-2694.
quadratic	$Performance = a + b + temp + c + temp^2$	Montagnes et al. 2008. Short-term temperature change may impact freshwater carbon flux: a microbial perspective. Global Change Biology 14.12: 2823-2838.
cubic	$Performance = a + b + temp + c + temp^2 + d + temp^3$	
quartic	$Performance = a + b + temp + c + temp^2 + d + temp^4 + e + temp^4$	

Appendix D Table 7: **Productivity GLMM.**

A. Estimated regression parameters, standard errors, *z*-values, *P*-values, and the R^2 value for the zero-inflated, negative-binomial GLMM for total offspring as a function of treatment, population, and temperature. Temperature was modeled as a quadratic and an interaction between the quadratic variable and treatment was included, as well as an interaction between population and treatment. Time effects and potential dependency between individuals within the same replicate was accounted for by a random term nested within treatment. **B**. Treatment estimates for the fixed values were back-transformed from the log scale. **C.** Random variance component estimates for replicate nested within treatment.

A. Treatment effects (log scale)

	Estimate ± *SE**	*z*-value	*P*-value	R^2
Intercept	-77.084 ± 8.845	-8.715	< *0.001*	0.764
Moderate treatment	-0.115 ± 0.139	-0.831	0.406	
Severe treatment	-1.091 ± 0.258	-4.235	< *0.001*	
Population Paluma	0.015 ± 0.082	0.186	0.852	
Temperature^2	-16.467 ± 1.802	-9.136	< *0.001*	
Temperature	2.816 ± 0.307	9.158	< *0.001*	
Moderate treatment:Population Paluma	-0.079 ± 0.136	-0.59	0.555	
Severe treatment:Population Paluma	0.441 ± 0.271	1.622	0.105	

B. Treatment estimates (back-transformed from log scale)*

Treatment	Population	Temperature^2	Temperature	response	*SE*	df	Lower *CI* (2.5%)*	Upper *CI* (97.5%)*
Control	Mt Lewis	1.15E-16	28	5.21	1.431	369	3.037	8.94
Moderate treatment	Mt Lewis	1.15E-16	28	4.64	1.386	369	2.582	8.35
Severe treatment	Mt Lewis	1.15E-16	28	1.75	0.649	369	0.844	3.63
Control	Paluma	1.15E-16	28	5.29	1.421	369	3.12	8.97
Moderate treatment	Paluma	1.15E-16	28	4.35	1.328	369	2.39	7.93
Severe treatment	Paluma	1.15E-16	28	2.76	0.828	369	1.531	4.98

C. Variance component estimates

Groups		Variance	*SD*	Correlation	
Replicate	(Intercept)	0.0164	0.128		
	Moderate	0.039	0.197	0.390	
	Severe	0.011	0.107	1.000	0.410

*The asterisk indicates the estimates were back-transformed from log scale because the ‘log’ link was used in the negative binomial GLMM. These estimates represent magnitudes of change (or multipliers) due to the ‘log’ link.

Appendix D Table 8: **Significance tests for differences in TPC parameters between heatwave treatments.**

A one-way ANOVA with a post hoc Tukey HSD test was used to assess deviances in TPC parameters (P_{max}, CT_{max}, T_{opt}, and B_{80}) between heatwave treatments.

			ANOVA		Tukey procedure post hoc analysis		
Trait	**Parameter**		**F-value**	**P-value**	**Estimate**	**SD**	**P-value**
Productivity	P_{max}	Treatment	62.527	*0.004*	-	-	-
		Intercept	-	-	64.065	1.968	-
		Moderate	-	-	*-15.904*	2.783	*0.022*
		Severe	-	-	*-31.116*	2.783	*0.003*
	Ct_{max}	Treatment	1.687	0.323	-	-	-
		Intercept	-	-	29.026	0.978	-
		Moderate	-	-	-0.090	1.383	0.998
		Severe	-	-	-2.245	1.383	0.364
	T_{opt}	Treatment	0.185	0.840	-	-	-
		Intercept	-		23.530	0.749	-
		Moderate	-		0.644	1.059	0.826
		Severe	-		0.343	1.059	0.945
	B_{80}	Treatment	5.291	0.104	-	-	-
		Intercept	-		3.715	0.835	-
		Moderate	-		2.869	1.181	0.998
		Severe	-		-0.777	1.181	0.364
Development speed	P_{max}	Treatment	3.732	0.153	-	-	-
		Intercept	-	-	0.123	0.003	-
		Moderate	-	-	-0.003	0.005	0.771
		Severe	-	-	-0.012	0.005	0.150
	Ct_{max}	Treatment	1.687	0.323	-	-	-
		Intercept	-	-	29.026	0.978	-
		Moderate	-	-	-0.090	1.383	0.998
		Severe	-	-	-2.245	1.383	0.364
	T_{opt}	Treatment	3.417	0.169	-	-	-
		Intercept	-	-	28.232	0.675	-
		Moderate	-	-	0.015	0.955	1.000
		Severe	-	-	-2.155	0.955	0.207
	B_{80}	Treatment	0.455	0.672	-	-	-
		Intercept	-	-	6.069	1.135	-
		Moderate	-	-	0.686	1.605	0.907
		Severe	-	-	-0.844	1.605	0.865
Wing size	P_{max}	Treatment	2.192	0.259	-	-	-
		Intercept	-	-	937.321	9.578	-
		Moderate	-	-	-22.955	13.546	0.341
		Severe	-	-	-25.907	13.546	0.280
	Ct_{max}	Treatment	1.687	0.323	-	-	-
		Intercept	-	-	29.026	0.978	-
		Moderate	-	-	-0.090	1.383	0.998
		Severe	-	-	-2.245	1.383	0.364
	T_{opt}	Treatment	2.968	0.195	-	-	-
		Intercept	-	-	20.526	0.395	-
		Moderate	-	-	0.126	0.559	0.973
		Severe	-	-	1.237	0.559	0.214
	B_{80}	Treatment	1.687	0.323	-	-	-
		Intercept	-	-	9.026	0.978	-
		Moderate	-	-	-0.090	1.383	0.998
		Severe	-	-	-2.245	1.383	0.364

Appendix D Table 9: **Development speed LMM.**

A. Analysis of deviance table to test the significant of main effects in the LMM on development speed. **B**. Estimated regression parameters, standard errors, *t*-values, *P*-values, and the R^2 value for the linear mixed-effects model for development speed as a function of treatment and temperature. Temperature was modeled as a quadratic. Time effects and potential dependency between individuals within the same replicate was accounted for by a random term nested within treatment. **C.** Random variance component estimate for replicate nested within treatment.

A. Analysis of deviance table (type II)

	Chisq	Df	Pr(>Chisq)
Treatment	0.8728	2	0.6464
Temperature^2	25.637	1	4.12E-07***
Temperature	54.675	1	1.42E-13***

B. Treatment effects

	Estimate ± *SE*	DF	*t*-value	*P*-value	R^2
(Intercept)	-0.2884 ± 0.053	181	-5.439	0.000	0.895
Moderate treatment	0.0004 ± 0.002	9	0.230	0.823	
Severe treatment	-0.0004 ± 0.002	9	-0.679	0.514	
Temperature^2	-0.0304 ± 0.006	181	-5.063	0.000	
Temperature	0.0163 ± 0.002	181	7.394	0.000	

C. Variance component estimates

	(Intercept)	Residual	Intraclass correlation
Standard deviation	0.003	0.002	0.585

Appendix D Table 10: **Wing size LMM.**

A. Analysis of deviance table to test the significant of main effects in the LMM on wing centroid-size. **B**. Estimated regression parameters, standard errors, *t*-values, *P*-values, and the R^2 value for the linear mixed-effects model for wing size as a function of treatment, population, and temperature. Temperature was modelled as a quadratic and an interaction between the quadratic variable and treatment was included, as well as an interaction between treatment and the linear term for temperature. Time effects and potential dependency between individuals within the same replicate was accounted for by a random term nested within treatment. **C.** Random variance component estimate for replicate nested within treatment.

A. Analysis of deviance table (type II)

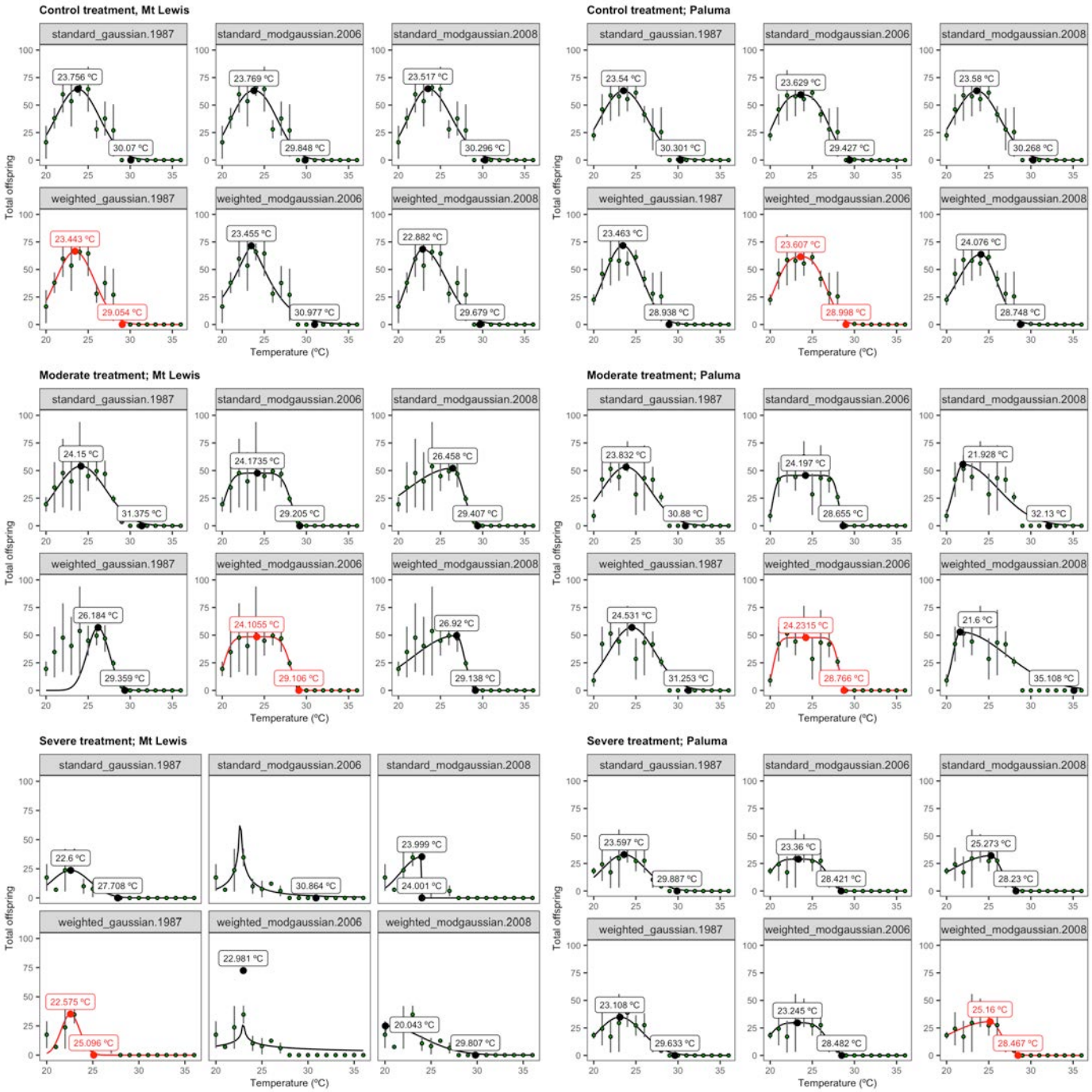
	Chisq	Df	Pr(>Chisq)
Population	71.559	1	2.20E-16***
Treatment	49.637	2	1.67E-11***
Temperature^2	70.698	1	2.20E-16***
Temperature	54.491	1	1.56E-13***

B. Treatment effects

	Estimate ± *SE*	DF	*t*-value	*P*-value	R^2
(Intercept)	-287.95 ± 400	528.0	-0.720	0.472	0.552
Population Paluma	24.39 ± 2	528.0	10.404	*0.000*	
Moderate treatment	295.27 ± 545	33.0	0.542	0.592	
Severe treatment	-1953.54 ± 625	33.0	-3.125	*0.004*	
Temperature^2	-158.14 ± 43	528.0	-3.653	*0.000*	
Temperature	49.35 ± 17	528.0	2.948	*0.003*	
Moderate treatment: Temperature^2	41.34 ± 59	528.0	0.702	0.483	
Severe treatment: Temperature^2	-212.25 ± 68	528.0	-3.105	0.002	
Moderate treatment: Temperature	-12.92 ± 23	528.0	-0.566	0.571	
Severe treatment: Temperature	80.76 ± 26	528.0	3.089	0.002	

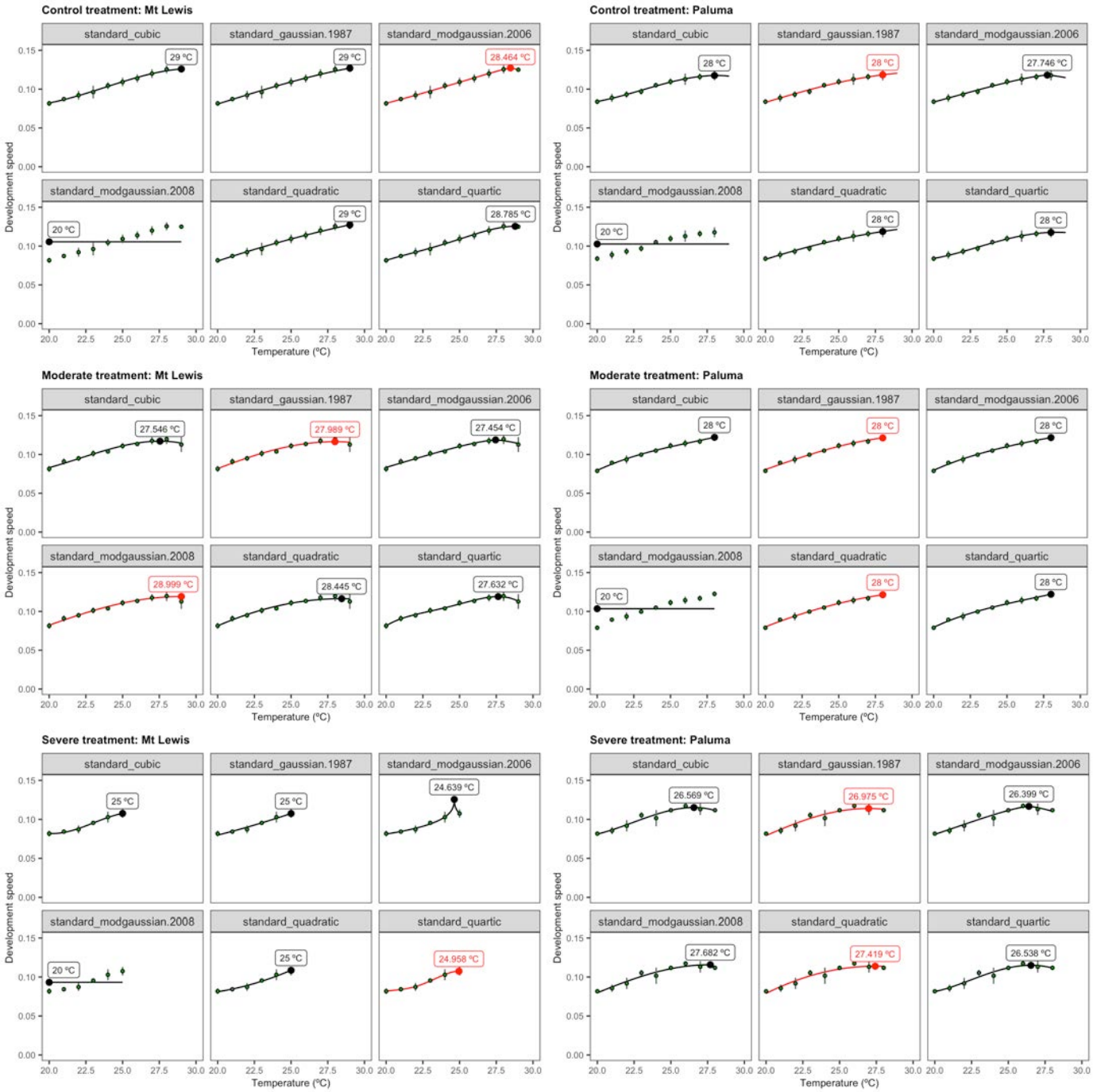
C. Variance component estimates

	(Intercept)	Residual	Intraclass correlation
Standard deviation	2.176	30.659	0.005



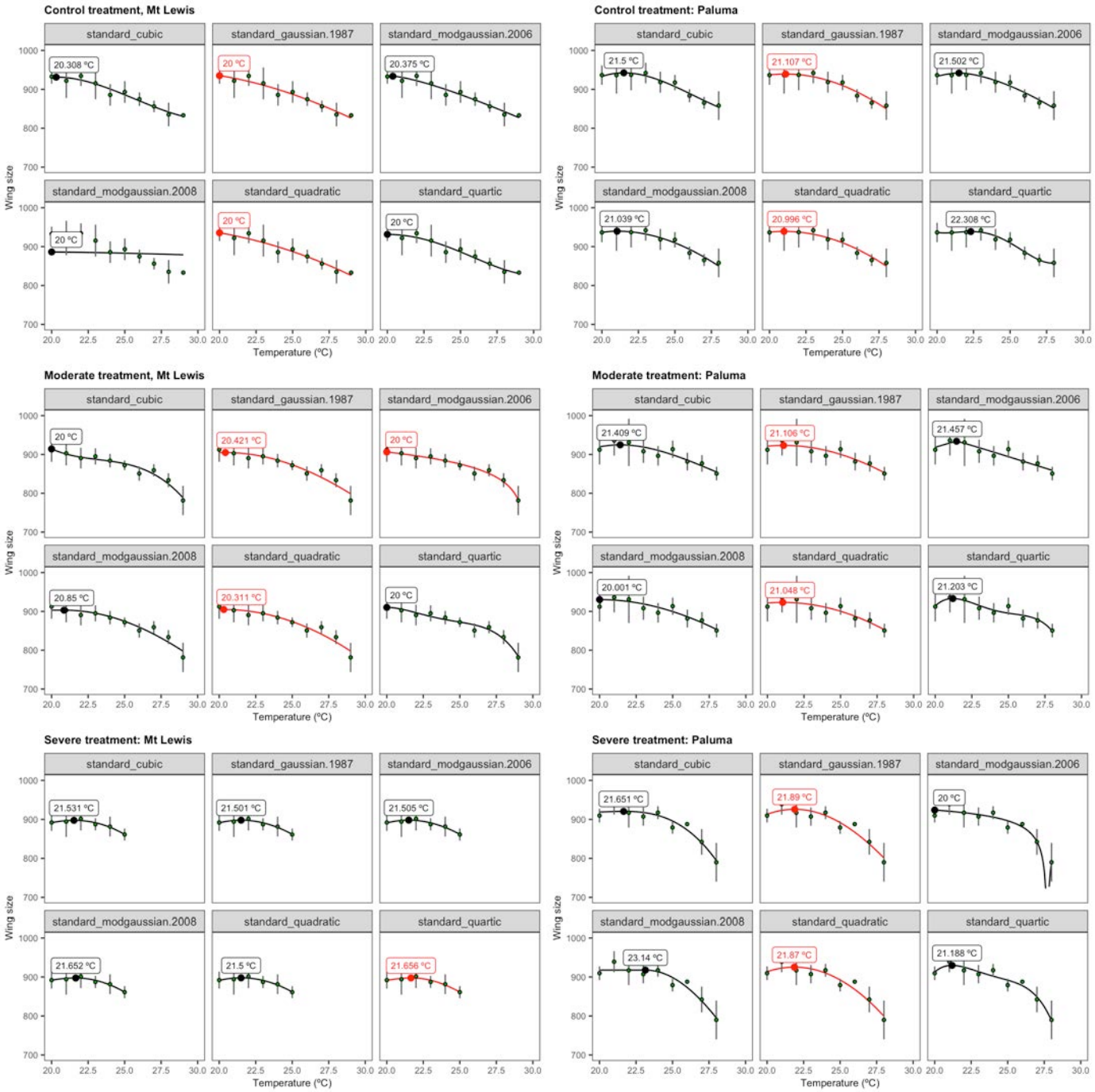
Appendix D Figure 1: Productivity non-linear least square model fit and selection.

Standard verse weighted nonlinear least square models for productivity of each treatment and population. Model shown in red is the best fit per AICc score and the model selected for parametrization. Weighted models were used due to heteroscedasticity of total offspring across temperature gradient.



Appendix D Figure 2: Development speed non-linear least square model fit and selection.

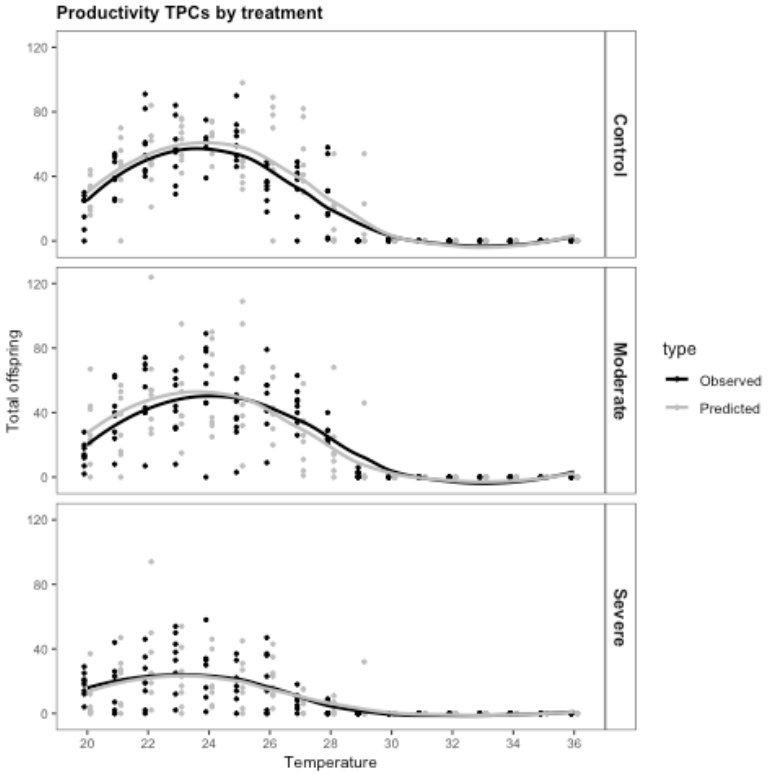
Standard nonlinear least square models for development speed of each treatment and population. Model(s) shown in red is the best fit per AICc score and the model selected for parametrization. If an AICc score was less than 2 from the best fit model, the model was included in model averaging. Non-weighted models were used instead of weighted because of homoscedasticity of errors of development speed and unequal sample sizes across temperature gradient.



Appendix D Figure 3: Wing size non-linear least square model fit and selection.

Standard nonlinear least square models for wing size of each treatment and population. Model(s) shown in red is the best fit per AICc score and the model selected for parametrization. If an AICc score was less than 2 from the best fit model, the model was included in model averaging. Non-weighted models were used instead of weighted because of unequal sample sizes across temperature gradient.

A



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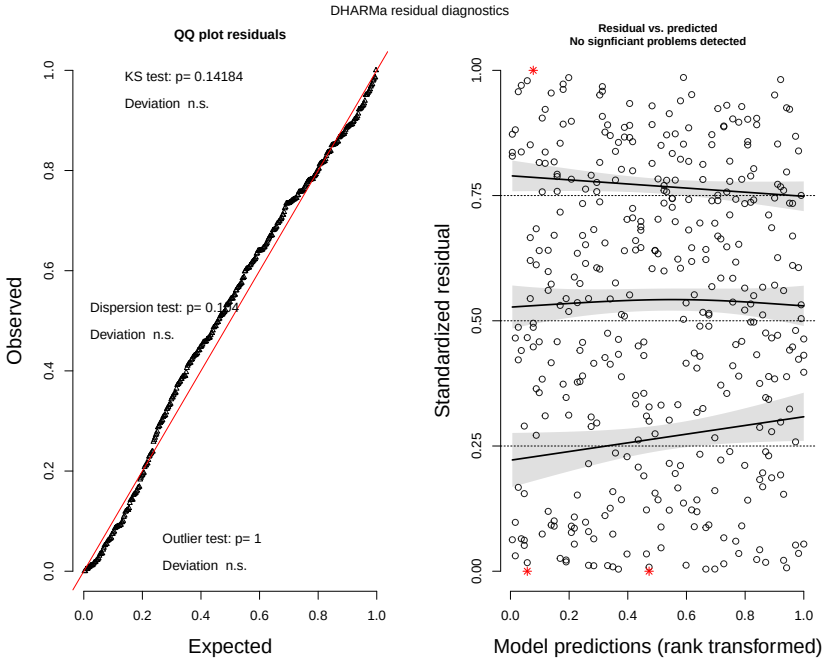
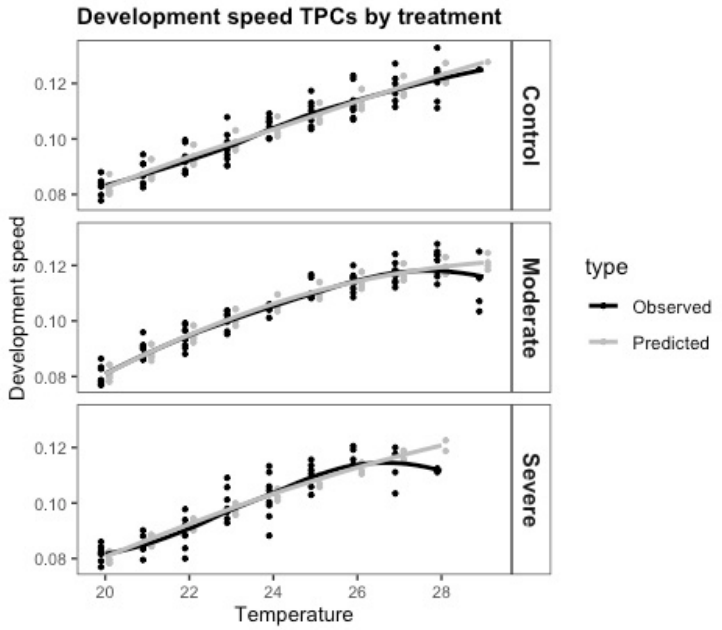


Appendix D Figure 4: Productivity GLMM fit.

A. Predicted values (grey) from the zero-inflated negative binomial GLMM compared to the raw data (observed; black) for each treatment. **B.** QQ-plot for standardized residuals and residuals verse model predictions for simulated residuals to check model fit. No deviations in residuals were detected against model predictions nor model parameters (both included and not included in the final model), and all tests of normality, independence, dispersion, and outliers indicated no significant problems.

A



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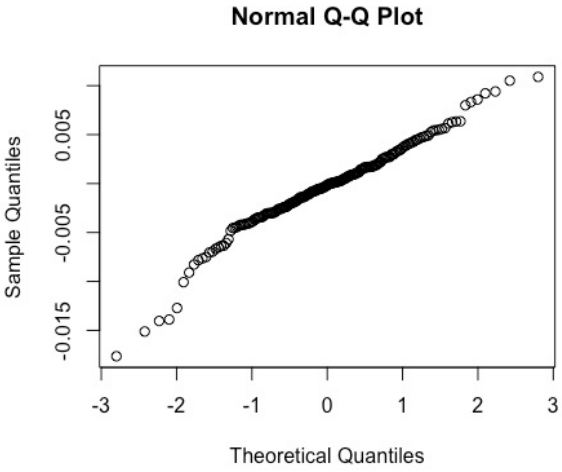

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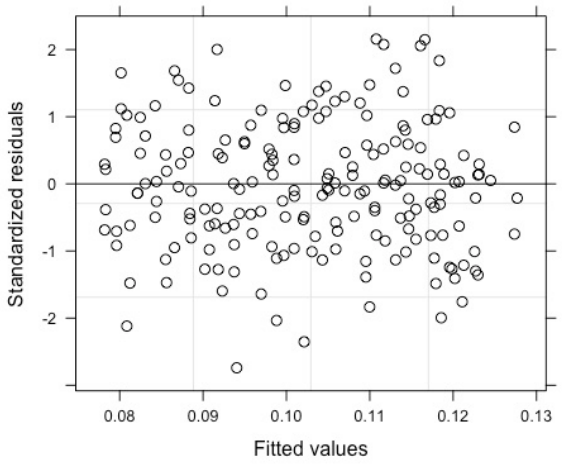
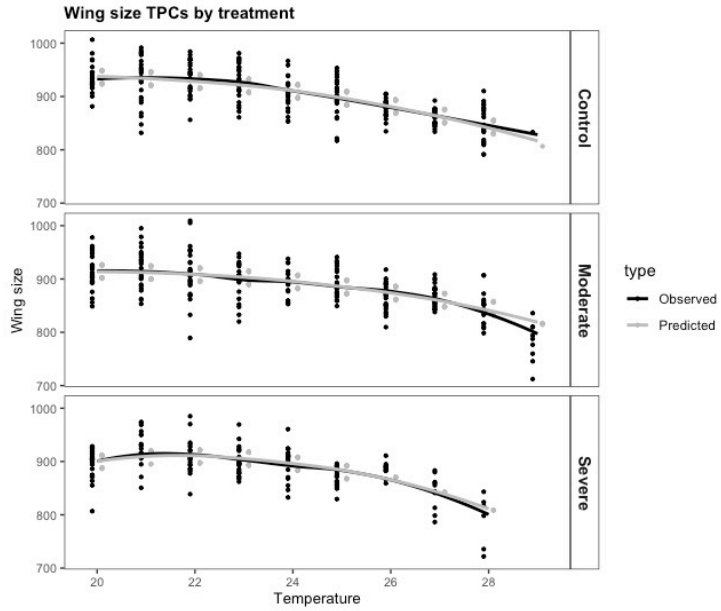


Appendix D Figure 5: Development speed LMM fit.

A. Predicted values (grey) from the LMM compared to the raw data (observed; black) for each treatment for development speed. **B.** QQ-plot for standardized residuals, and, **C.** residuals verse model predictions to check model fit. No deviations in residuals were detected against model predictions nor model parameters (both included and not included in the final model), and all tests of normality, independence, dispersion, and outliers indicated no significant problems.

A



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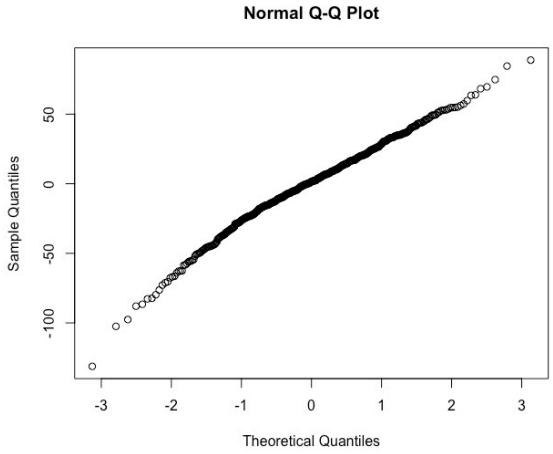

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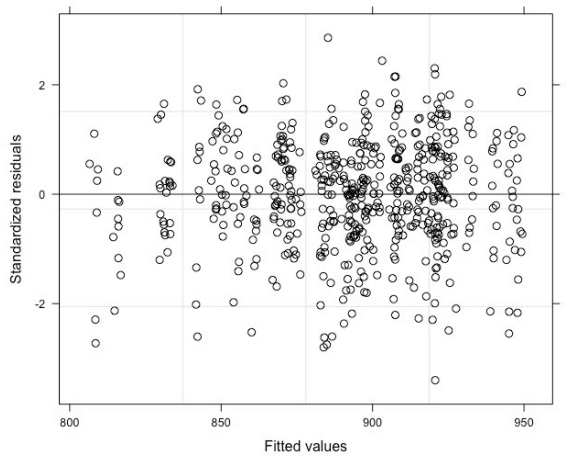

Appendix D Figure 6: Wing size GLMM fit.

A. Predicted values (grey) from the LMM compared to the raw data (observed; black) for each treatment for wing size. **B.** QQ-plot for standardized residuals, and, **C.** residuals verse model predictions to check model fit. No deviations in residuals were detected against model predictions nor model parameters (both included and not included in the final model), and all tests of normality, independence, dispersion, and outliers indicated no significant problems.