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# Thermal adaptation of life history traits in the Drosophila melanogaster group

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A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

# THERMAL ADAPTATION OF LIFE HISTORY TRAITS IN THE *DROSOPHILA MELANOGASTER* GROUP

(Thesis format: Integrated Article)

by

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# Abstract

Thermal adaptation is typically detected by examining the tolerance to extreme temperatures in a few populations within a single life stage. However, the extent to which adaptation occurs among many different populations might depend on the tolerance of multiple life stages and the average temperature range that the population experiences. Here, I examined adaptation to local temperature conditions in four species of fruit flies, including a cosmopolitan species, *Drosophila melanogaster*, and three species with geographically small-sized ranges, *D. nepalensis*, *D. sechellia*, and *D. mauritiana*. The cosmopolitan species showed adaptation to native temperatures during the larval and adult life stages, but the species with geographically restricted ranges differed in their responses to temperature changes during all life stages. Therefore, species with restricted ranges are more sensitive to temperature shifts than widespread species, and within species there are differences in tolerance among populations and life stages.

## Keywords

Mating behaviour, local adaptation, phenotypic plasticity, climate change

# Co-Authorship Statement

Christopher Austin completed all data collection, analysis, and wrote each chapter. Amanda Moehring edited all drafts of this thesis and contributed to the ideas and conclusions of each chapter.

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# List of abbreviations

3D: Three dimensional

ANODEV: Analysis of deviance

GLZ: Generalized linear model

HSP: Heat shock protein

kya: One thousand years ago

mya: One million years ago

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# Chapter 1

# 1 Introduction

Abiotic factors such as humidity, pressure, and temperature can affect the survival and reproduction of organisms. When conditions exceed certain thresholds, survival or reproduction is impaired, leading to reduced fitness (Parsons 1979; Marchand and McNeil 2000; Huey and Berrigan 2001). The optimum level and range of an abiotic factor can be determined by measuring the fitness of many different individuals from a single population across a range of values of the abiotic factor of interest (Huey and Berrigan 2001). Strictly speaking, fitness is measured by lifetime reproductive success. However, the related metric of survival to reproductive age is often used instead since it is a relatively easy trait to measure, and is an important component of fitness that is affected by many abiotic factors, including temperature. Temperature affects animals in every habitat on Earth by reaction rates and the stability of molecules. As a result, the effect of extreme temperatures on the physiology, ecology, and distribution of organisms has been studied intensely (Cossins and Bowler 1984; Hochachka and Somero 2002; reviewed by Angilletta 2009).

## 1.1 Insect responses to temperature changes

### 1.1.1 Immediate and long-term responses to temperature changes

Most insects are ectotherms. The body temperatures of ectotherms follow the ambient temperature (Angilletta 2009). Insects cannot tolerate an infinite range of temperatures because many molecular components of the organism, such as membranes, proteins, and carbohydrates, become unstable and degrade at temperatures beyond a certain range (Angilletta 2009). Therefore, insects must remain in an environment that has suitable temperatures; otherwise, their body temperature might exceed the upper or lower limit that the organism can tolerate. Insects are able to respond to small changes in temperature through mechanisms such as modifying their behaviour or physiology. These changes can happen on different timescales, from seconds, to hours, or even days. Therefore,

responses to temperature variation are categorized either as acute, which occur immediately after exposure, or chronic, which occur after long-term exposure to an increase or decrease in temperature (Tattersall *et al.* 2010). These phenotypic responses can be measured across a range of temperatures, and this relationship between temperature and a phenotypic trait is known as a thermal reaction norm (Kingsolver *et al.* 2004).

#### 1.1.1.1 Acute response to temperature changes in insects

Insects first respond to a change in temperature through an acute response. One example of an acute response is a change in metabolic rate, which occurs due to the effect of changing temperatures on chemical reaction rates and kinetic energy (Hochachka and Somero 2002; Tattersall *et al.* 2010). As temperatures rise above the preferred temperatures of insects, there is an increase in metabolic rate (Neven 2000). As temperatures continue to increase above the preferred temperatures, the metabolic rate first reaches a maximum, then rapidly drops, often immediately followed by death (Neven 2000). Before this lethal temperature is reached, a critical thermal limit exists where, within a range of temperatures, insects are able to reverse the effects of acute heat stress and return to normal conditions (Neven 2000; Angilletta 2009). As would be expected, true ectotherms also experience a drop in metabolic rate as temperatures decrease from their preferred temperatures. In addition to the effect of a change in temperature on metabolic rates, there are other acute effects such as faster or slower development (Trotta *et al.* 2006; Austin and Moehring 2013) and increased or decreased rates of locomotion (Gibert *et al.* 2001; Angilletta *et al.* 2002) at increased or decreased temperatures, respectively.

#### 1.1.1.2 Phenotypic plasticity in insects as a response to chronic changes in temperature

After a chronic exposure to a change in temperature, insects respond through thermal phenotypic plasticity. Phenotypic plasticity is the ability of a particular genotype to be differentially expressed depending on the environment in which the organism lives. Therefore, phenotypic plasticity allows individuals to adjust their phenotype temporarily

or permanently to the environment in which they currently reside (David 2004; Angilletta 2009; Austin & Moehring 2013). One way that insects can use phenotypic plasticity to adjust their physiology to their current environment is through acclimatization (Angilletta 2009). Acclimatization is the physiological change that is associated with a chronic change in the natural environment of an organism, where many environmental factors are changing at once (Bullock 1954; Hochachka and Somero 2002; Tattersall *et al.* 2010). The effect of an individual component of the environment (such as temperature) on an organism is called acclimation. Acclimation is only observed in a controlled laboratory environment because various environmental factors can be held constant to isolate the physiological effects of a single factor of interest on the survival or performance of an individual (Hochachka and Somero 2002; Tattersall *et al.* 2010).

Many researchers assume that phenotypic plasticity evolved as a mechanism to increase fitness. This is known as the "beneficial acclimation hypothesis" (Kristensen *et al.* 2008; Angilletta 2009; Cooper *et al.* 2010). However, this hypothesis has been debated in the literature, as organisms often adjust incorrectly to their environment and as a result might suffer fitness consequences (Huey and Berrigan 1996; Huey *et al.* 1999; Wilson and Franklin 2002; Angilletta 2009; Cooper *et al.* 2010). For example, *Drosophila melanogaster* flies reared at a warm temperature were predicted to walk faster when tested at warm temperatures compared to flies that were reared at a cold temperature. However, flies that were reared at intermediate temperatures walked faster at all temperatures compared to flies reared at both warm and cool environments, which does not support the beneficial acclimation hypothesis because the acclimation treatment did not maximize performance at the rearing temperatures (Gibert *et al.* 2001). Therefore, phenotypic plasticity might not always increase the fitness of organisms in their natural environment.

There are two main types of phenotypic plasticity. The first type is reversible phenotypic plasticity and is also called phenotypic flexibility (Hazel 1995; Seebacher 2005). Reversible phenotypic plasticity allows individuals to temporarily adjust their physiology to chronic temperature changes in their immediate environment, for example, through changes in membranes or the production of different isozymes (Hazel 1995; Baldwin and

Hochachka 1970; Tattersall *et al.* 2010). Reversible phenotypic plasticity is thought to be beneficial because it allows organisms to avoid the detrimental effect of extreme temperatures on fitness (Tattersall *et al.* 2010). Phenotypic plasticity can also be irreversible or fixed, as is seen in the changes made during development in response to the thermal environment experienced during rearing. These changes are fixed for the remainder of the insect's life. Some examples of developmental phenotypic plasticity are increased body size when insects are reared in cooler temperatures (Angilletta 2009; Tattersall *et al.* 2010; Austin and Moehring 2013) and increased desiccation resistance when reared in dry environments (Bubliy *et al.* 2012; Parkash *et al.* 2012).

### 1.1.2 Evolutionary adaptation of thermal tolerance in insects

In addition to acute and chronic physiological responses, the underlying genetic basis of physiology can also evolve over successive generations (Tattersall *et al.* 2010), potentially leading to adaptation to their environment. An adaptation is a heritable characteristic that evolves through natural selection and results in an increase in fitness (Dobzhansky *et al.* 1968; Kawecki and Ebert 2004). Thermal adaptation occurs when individuals that are able to thrive in a particular thermal environment pass on that ability to their progeny, causing an increase in frequency of the genes involved (Kawecki and Ebert 2004; Angilletta 2009). There are many examples of thermal adaptations: cellular membrane stability after a change in temperature (Hochachka and Somero 2002; Overgaard *et al.* 2008; Angilletta 2009), proteins known as heat shock proteins (HSP) that help refold denatured proteins at extreme temperatures (Hochachka and Somero 2002; Tattersall *et al.* 2010; Carmel *et al.* 2011), and behavioural adaptations that allow organisms to either avoid or tolerate changes in temperature (Angilletta *et al.* 2002; Dolgin *et al.* 2006; Le Lann *et al.* 2011). Adaptation to local habitat conditions can be detected among many populations or species. For example, two species of Coleoptera were found to outperform each other in their native environments, which differ in mean temperature by only 4 °C (Blumberg 1971). These species are thus adapted to the local thermal conditions that they experience (Blumberg 1971). A large number of studies have also investigated adaptation in two particular species of *Drosophila*, *D. melanogaster* and *D. simulans*, because they make a suitable model system to study the effects on

adaptation between two close relatives with similar cosmopolitan ranges (reviewed by David *et al.* 2004).

#### 1.1.2.1 Local adaptation to native temperatures

Local adaptation is the genetic specialization of populations over evolutionary time to the unique environmental characteristics of the place where they live. Locally-adapted populations have higher fitness within their native environment, but lower fitness in other environments (Kawecki and Ebert 2004; Angilletta 2009). To become locally adapted to an environment, populations evolve traits that make them more fit under local conditions, providing an advantage over populations from other locations that do not experience the same conditions (Kawecki and Ebert 2004). Gene flow and maintenance of high intra-population genetic variation can oppose the process of local adaptation because they impede local genetic specialization to current conditions (Kawecki and Ebert 2004; North *et al.* 2011). High intra-population genetic variation for thermal tolerance is initially required for local adaptation. As populations become locally adapted, the genetic variation is removed by selection.

To detect local adaptation, the fitness of resident populations in their native environment must be greater, on average, than the fitness of non-resident populations in that same environment (Kawecki and Ebert 2004). Local adaptation is detected experimentally when there is a significant genotype-by-environment interaction for traits related to fitness, and when residents outcompete non-residents in their local conditions (Kawecki and Ebert 2004). Local adaptation is considered to occur only when both of these criteria are met; otherwise, genetic drift could account for the variation among strains (Kawecki and Ebert 2004). In certain situations, genetic drift can allow for specialization to local conditions, which might lead to populations appearing locally adapted even if natural selection did not lead to adaptation to local conditions. While adaptation to local conditions is generally favourable, individuals from locally-adapted populations might suffer if they are unable to respond when conditions change in their environment.

#### 1.1.2.2 Consequences of local adaptation in insects

Climate change is predicted to have adverse effects on the survival and reproduction of many species (Intergovernmental Panel on Climate Change 2007). Indeed, evidence suggests that many species have already shifted their range in response to climate change, with the most recent estimate suggesting that terrestrial species are shifting 16.9 km poleward per decade (Chen *et al.* 2011). Populations of insects that are present across a relatively small area and whose ancestors are continually under thermal selection might have a narrow range of temperatures in which they can survive and reproduce. If local temperatures increase, as predicted by climate change, individuals from locally-adapted populations might no longer be able to persist in their current habitat (Chown *et al.* 2010; Parkash *et al.* 2013). For example, if species that have enzymes that only function optimally across a narrow range of temperatures are faced with changing ambient temperatures, they might not survive, while others with wide breadths might be able to adjust to these changing conditions (Baldwin and Hochachka 1970). If only a few individuals survive, they might not be able to find mates or the population might become inbred (Hoffmann and Parsons 1991). If no individuals live to reproduce, the population will become extirpated (Hoffmann and Parsons 1991); if this happens across the entire range of the species, then the species could become extinct. Alternatively, a shift in temperature might represent a selection event leading to adaptation to warmer temperatures.

Individuals that are subjected to changing temperatures in their natural environment must either move to a different location to track suitable temperature ranges (Hill *et al*. 2011) or adapt to the new thermal environment, provided there is genetic variation that selection can act on; otherwise, the population will be extirpated (Parkash *et al.* 2013). In addition to genetic adaptation, those individuals able to exhibit phenotypic plasticity can potentially adjust during development to cope with changes in temperature. Substantial documentation in the literature of the potential effects of climate change on the range boundaries of species suggests that many species are sensitive to changes in their thermal environment (Hughes 2000; Angilletta 2009; Hill *et al.* 2011; Parkash *et al.* 2013). For example, a cold-adapted species of *Drosophila, D. nepalensis,* has increased its altitude

to track cooler temperatures (Parkash *et al.* 2013; Chapter 3). In addition, within the last 100 years, over 63% of European butterfly species have shifted their range northward, with fewer than 3% shifting southward (Parmesan *et al.* 1999).

One limitation to the evolution of insects to a changing thermal environment is that many tropical species are already living close to their upper thermal limit (Addo-Bediako *et al.* 2000; Deutsch and Tewksbury 2008; Somero 2010). Any increases in mean temperature might result in extirpation and eventual extinction (Addo-Bediako *et al.* 2000). Therefore, many species are highly susceptible to extirpation in the face of climate change if they are not able to disperse or rapidly adapt to a new thermal environment. However, those individuals that can use phenotypic plasticity to adjust their phenotype might be able to survive ongoing climate change (Stillman 2003; Somero 2010).

## 1.2 Thermal biology of the genus *Drosophila*

Thermal adaptation has been well investigated in *Drosophila* using multiple metrics, including cold and heat tolerance and specialization to intermediate temperatures (Hoffmann *et al.* 2003). The genus *Drosophila* has almost 2 000 described species that inhabit a wide variety of terrestrial environments (Guruprasad *et al.* 2010), and many of these species have been used in studies of thermal adaptation. The focus of most studies has been on the well-known laboratory organism, *D. melanogaster*. However, other species within the *melanogaster* species subgroup have also been the object of comparative studies of thermal adaptation and phenotypic plasticity (Hoffmann *et al.* 2003; Matute *et al.* 2009; Nyamukondiwa *et al.* 2011; Overgaard *et al.* 2011; Strachan *et al.* 2011; Kellermann *et al.* 2012; Austin and Moehring 2013).

### 1.2.1 Evolutionary history of *Drosophila*

The evolutionary history of *Drosophila* is complex given the number of species and the geographic scale in which they are found. Throckmorton (1975) suggested that the genus *Drosophila* originated from the tropical areas of the Old World based on evidence from the current distribution of *Drosophila* species. Within the genus *Drosophila*, the *melanogaster* subgroup contains only nine species: *D. erecta, D. orena, D. yakuba, D.*

*santomea, D. teissieri*, *D. sechellia*, *D. mauritiana*, *D. simulans*, and *D. melanogaster* (Figure 1.1). All of these species share a common ancestor from Africa (David *et al.* 2007; Marygold *et al.* 2013), which speciated into many descendants that subsequently migrated throughout the world (David *et al.* 2007; Marygold *et al.* 2013). The common ancestor lived in a tropical African climate approximately 3.4 – 3.5 million years ago (mya), based on the most recent study of divergence time of the *melanogaster* subgroup (Obbard *et al.* 2012).

## 1.3 Approaches to study the thermal biology of *Drosophila*

Many approaches have been used to study the thermal biology of *Drosophila*, including clinal and non-clinal studies within species, as well as thermal adaptation studies among species (reviewed by Hoffmann *et al.* 2003). Populations have been compared along a natural gradient, usually a geographic cline, along which temperature varies. A comparison of various traits measured across populations of the same species that are experiencing incremental differences in temperature can allow for an assessment of local adaptation to native temperatures (Hoffmann *et al.* 2003; Sarup *et al.* 2009). For example, altitudinal clines for local adaptation to native temperatures have been examined in *D. buzzatii* found on mountain ranges in the Canary Islands (Sarup *et al.* 2009). As altitude increases and local temperature generally decreases, populations of *D. buzzatii* had decreased heat tolerance (Sørensen *et al.* 2005). There are also clines for chromosomal arrangements that affect the thermal tolerance of *D. robusta* in the Smoky Mountains of the Eastern USA. These clines are found along both altitudinal and latitudinal gradients (Etges 1989). In *D. subobscura*, alleles that are found in populations from warm climates have spread to all populations examined, except one. This spread of greater tolerance to high temperatures is thought to be the result of the increase in mean temperatures (Balanyá *et al.* 2006).

Non-clinal differences can also be used to study genetic adaptation as long as enough independent points are included to draw strong conclusions (Hoffman *et al.* 2003). Non-clinal studies have examined thermal adaptation in *Drosophila* by comparing the

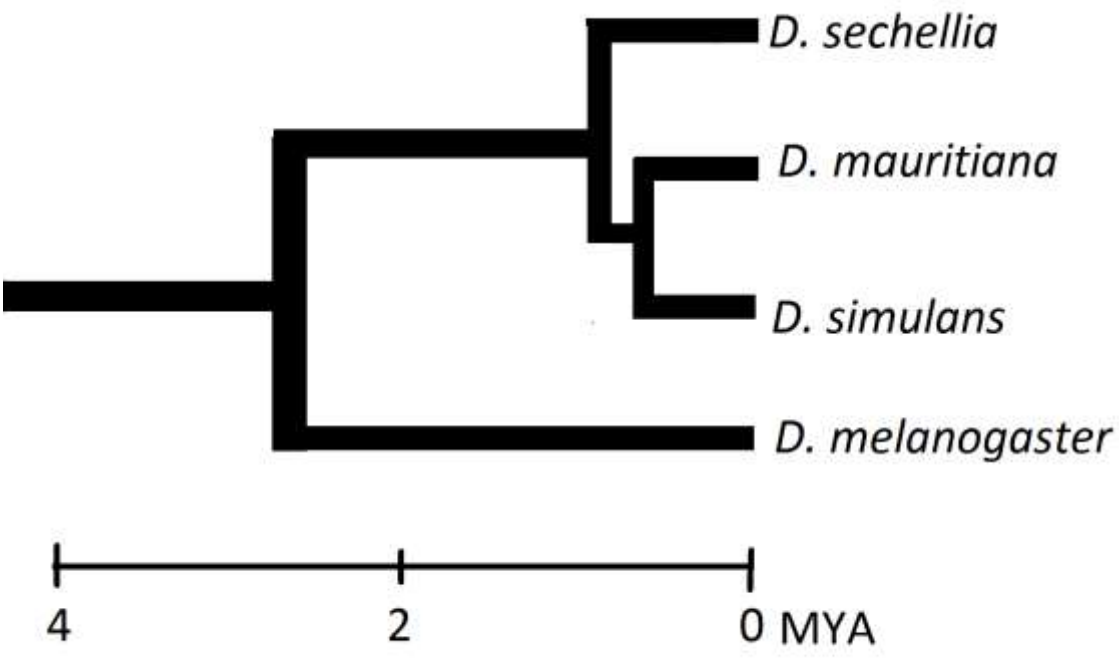


**Figure 1.1** A scaled phylogenetic tree depicting four species in the *D. melanogaster* species subgroup. The scale shows the approximate divergence time based on data from Lachaise and Silvain (2004). Redrawn from David *et al.* (2007).

differences in thermal tolerance between temperate and tropical locations of a single species of *Drosophila*, or between populations exposed to different temperature conditions in the laboratory for multiple generations. For example, *D. subobscura* flies sampled from colder areas were more tolerant to cold stress than flies from warmer areas (David *et al.* 2003). In addition, *D. serrata* flies maintained in the laboratory for multiple generations under different temperature conditions showed higher tolerance for cold stress events if they came from the cold-climate populations (Magiafoglou and Hoffmann 2003).

Finally, other studies have examined thermal adaptation by comparing species of *Drosophila*. Kellermann *et al.* (2012) showed that for 94 *Drosophila* species the upper thermal limits vary less than their lower thermal limits, indicating that an evolutionary response to warmer temperatures may be more constrained than a response to colder temperatures. Two studies separately compared the heat and cold tolerance of the same 18 species of *Drosophila*, before and after acclimation, and found that both heat and cold tolerance can be increased through phenotypic plasticity, regardless of the sampling location and species (Nyamukondiwa *et al.* 2011; Strachan *et al.* 2011). Matute *et al.* (2009) showed that two species of *Drosophila* found at different altitudes on a single island are reproductively isolated in response to differences in temperature preference. In summary, thermal adaptation has been investigated in the genus *Drosophila* in many ways, all of which provide insight into how species are adapting to their native environment.

### 1.3.1 Thermal biology in the *D. melanogaster* species subgroup

#### 1.3.1.1 Thermal biology of *D. melanogaster*

The majority of studies of thermal adaptation in *Drosophila* have focused on the well-known genetic model, *D. melanogaster*, with fewer studies examining its close relative, *D. simulans* (reviewed by David *et al.* 2004). The powerful genetic tools available in *D. melanogaster* have made it appealing as a research organism. However, there are limitations to studying the thermal biology of *D. melanogaster*. The species lives in close

association with humans. Researchers often recover the flies near or even inside buildings inhabited by humans. The commensal nature of *D. melanogaster* has allowed for the species to be transported worldwide, in both temperate and tropical locations (Guerra *et al.* 1997; David *et al.* 2004), which can complicate studies of thermal adaptation, as the sampled fly might not be genetically adapted to the conditions in which they were collected (David *et al.* 2004).

Despite the problems of human commensalism, *D. melanogaster* has been used in a number of studies comparing differences in the thermal biology of specimens collected both within and between continents. In general, there is clear variation among strains in their response to changing temperatures. A comparison of heat and cold stress in populations of *D. melanogaster* sampled from Italy and Denmark to those from the Canary Islands and Mali found that tropical flies had a higher tolerance to heat compared to the temperate flies (Guerra *et al.* 1997). Local adaptation to native temperatures has also been detected along latitudinal clines in Eastern Australia, Europe, and South America (Hoffmann *et al.* 2002; Trotta *et al.* 2006). Genetic differences in thermal adaptation can also be detected on extremely small scales such as on opposite sides of a mountain range, as seen in populations of *D. melanogaster* in 'Evolution Canyon' in Israel that are only separated by several hundred meters (Nevo *et al.* 1998). However, the repeatability of these studies has been questioned due to varying amounts of inbreeding among different seasons (Nevo *et al.* 1998; Drake *et al.* 2005; Rashkovetsky *et al.* 2006). The ability of *D. melanogaster* to adapt rapidly to temperature changes might partially explain the worldwide range of *D. melanogaster*. Variation in thermal tolerance in *D. melanogaster* is best described by the climatic metric of the mean temperatures of the warmest and coldest months for high and low temperature adaptation, respectively (Hoffmann *et al.* 2002).

Another way that thermal adaptation has been examined in *D. melanogaster* is by studying the adaptation, that is, specialization to rearing temperatures in the laboratory through maintenance or selection experiments (Partridge *et al.* 1995). Researchers maintained separate colonies of *Drosophila* for many generations under different thermal conditions and then examined them to see if adaptation has occurred. *D. melanogaster*

seems to adapt quickly to a new thermal environment and shows differences in survival when presented with heat stresses (Cavicchi *et al.* 1995). The capacity to adapt in the laboratory suggests that if natural populations are exposed to changing conditions in their environment, they are able to adapt to these changes. One study examined mating success after maintenance at either 18 or 25 °C and found that males had higher mating success at their maintenance temperature compared to males maintained at other temperatures (Dolgin *et al.* 2006), a finding that is consistent with the beneficial acclimation hypothesis. The results of these studies provide further support to the theory that *D. melanogaster* can adapt rapidly to new thermal environments.

Many of the studies of thermal adaptation in *D. melanogaster* focus on a single life stage, but extreme temperatures can potentially have detrimental effects on survival at one life stage while not affecting another. During the early larval stages, *D. melanogaster* larvae preferred warmer temperatures by choosing a particular thermal habitat that facilitates more rapid developmental times. However, the later stages of larvae preferred cooler temperatures, potentially to provide an advantage during the subsequent immobile pupal stage (Dillon *et al.* 2009). A recent study showed that in the genus *Drosophila*, adults across most of the species tested are more phenotypically plastic with respect to cold exposure than are larvae of the same species (Mitchell *et al.* 2013). Considering the entire life cycle is therefore important when drawing conclusions about thermal adaptation in a particular species.

The measure of fitness used varies among studies of *D. melanogaster*, which makes it difficult to compare results directly. Ideally, the fitness of an organism is measured as lifetime reproductive success. However, it is not always feasible to measure lifetime reproductive success, and so alternative measures of fitness are often used. In studies of thermal adaptation in *Drosophila*, fitness is most often measured by the following: temperature preference, which can mediate the effects of extreme temperatures and therefore increase survival (Dillon *et al.* 2009); reproductive output ($R_0$), survival (Nyamukondiwa *et al.* 2011), or intrinsic rate of population increase (r; Dillon *et al.* 2007; Marshall and Sinclair 2010). While these all act as proxy measures of fitness, each of these measures contributes directly to fitness through either survival or reproduction,

and so are worthwhile metrics for studying the effect of a range of temperatures on the fitness of *D. melanogaster*.

#### 1.3.1.2 Thermal biology of *D. simulans*

Fewer studies of thermal adaptation have been conducted on the close relative of *D. melanogaster*, *D. simulans*. The common ancestor to these species originated in tropical Africa approximately 3 million years ago (mya) by recent estimates based on neutral substitution rates (Lachaise and Silvain 2004; Figure 1.1). However, other studies have estimated the divergence time to be as recent as 360 thousand years ago (kya; Cutter *et al.* 2008). Interestingly, *D. simulans* is also found nearly worldwide, but is absent from large parts of some continents, for example, in Eastern Asia, with no explanation to date (David *et al.* 2004). Unlike *D. melanogaster*, *D. simulans* is rarely found inside the houses of humans (Capy and Gibert 2004). *D. simulans* only colonized the New World within the last five hundred years and has much lower genetic diversity among New World populations compared to Old World populations (Irvin *et al.* 1998). *D. simulans* forms clines for heat and cold tolerance (David *et al.* 2004; Arthur *et al.* 2008). It is not well adapted to high temperatures across the entire life cycle, although the adult life stage can tolerate high temperatures (Murphy *et al.* 1983; David *et al.* 2004; Austin and Moehring 2013). For example, *D. simulans* was found to have a decrease in performance for multiple life history traits as temperatures above or below 24 °C (Austin and Moehring 2013).

#### 1.3.1.3 Comparison of the thermal biology of *D. melanogaster* and *D. simulans*

*D. melanogaster* and *D. simulans* have been the focal species of many comparative studies of thermal adaptation (reviewed by David *et al.* 2004). These species are cosmopolitan and closely related, yet have different evolutionary histories. Because of these factors, they are often used as a model to test species differences in the ability to tolerate both heat and cold stresses, as well as optimum temperature (reviewed by David *et al.* 2004). However, many of these studies only compare single populations of each

species. The populations may not be representative of the entire species distribution as there might be substantial differences among populations within each species. Multiple traits that have been compared among populations—including ovariole number, mass, desiccation and starvation tolerance, development duration, and allozymes—show that *D. melanogaster* is much more variable across populations compared to *D. simulans* (Irvin *et al.* 1998; Chakir *et al.* 2002; Capy and Gibert 2004; David *et al.* 2004). This variation suggests that separate geographic populations of *D. melanogaster* are much more genetically differentiated compared to populations of *D. simulans*, and thus may be more locally adapted.

With respect to temperature, *D. melanogaster* has been shown to tolerate a wider range of temperatures compared to *D. simulans* across several different populations (Mckenzie 1978; Capy *et al.* 1993; David *et al.* 2004). However, *D. simulans* is sometimes able to tolerate higher or lower temperatures than *D. melanogaster* in some locations (Tantawy and Mallah 1961; Mckenzie 1978; Schnebel and Grossfield 1984; Krstevska and Hoffmann 1994; Pétavy *et al.* 2001; Chakir *et al.* 2002; reviewed by David *et al.* 2004). In a comparative study of the optimum temperature of the two species from two geographic locations, *D. melanogaster* had an overall wider tolerance and a warmer optimum temperature than *D. simulans* (Pétavy *et al.* 2001). Another study, using flies collected over a 20° latitudinal range showed a cline for cold tolerance in *D. melanogaster*, whereas *D. simulans* did not form a cline for cold tolerance (Davidson 1990). In general, *D. simulans* had only weak or completely absent clinal patterns, whereas strong clinal patterns are observed for *D. melanogaster* (Hoffmann *et al.* 2002; Arthur *et al.* 2008).

One hypothesis for the greater differentiation among populations of *D. melanogaster* than *D. simulans* is that *D. melanogaster* is able to overwinter, whereas *D. simulans* is not (Boulétreau-Merle *et al.* 2003; Schmidt, pers. comm.). Populations of *D. melanogaster* that are from colder regions might evolve tolerance to cooler temperatures experienced over winter. In contrast, *D. simulans* is thought to have an annual spring migration from warmer locations, which might result in a lower tolerance to colder temperatures among all populations of *D. simulans* (Boulétreau-Merle *et al.* 2003; P. Schmidt and E.

Behrman, pers. comm.). However, most of these studies drew their conclusions from one or two populations and might not represent both species across their entire distribution (David *et al.* 2004).

#### 1.3.1.4 Thermal biology of *D. mauritiana* and *D. sechellia*

The thermal tolerance of other species in the *D. melanogaster* species subgroup has not been studied as extensively as in *D. melanogaster* and *D. simulans*. However, some of those species, for example *D. mauritiana* and *D. sechellia*, provide an interesting contrast to *D. melanogaster* and *D. simulans* because they are evolutionarily closely related to these two species but are restricted to island habitats (David *et al.* 2007). In addition, *D. sechellia* and *D. mauritiana* can each hybridize and produce fertile offspring with *D. simulans,* which makes them a useful genetic system for studying thermal tolerance (Lachaise *et al.* 1986). The lack of full reproductive isolation might be due to insufficient isolation time—approximately 250 kya for both *D. mauritiana* and *D. sechellia* from the mainland species, *D. simulans* (McDermott and Kliman 2008).

*Drosophila mauritiana* and *D. sechellia* are restricted to the small Indian Ocean islands of Mauritius and the Seychelles, respectively. They experience a relatively constant climate compared to temperate populations of *D. melanogaster* and *D. simulans* (Mauritius Meteorological Services 2013; Ministry of Environment and Energy 2013). This difference in amounts of temperature variation is reflected in the number of functional heat shock protein (*hsp*) genes, whose products protect the organism from heat stress, that are present in tropical *vs.* temperate species in the subgroup. In gene duplication events, if both gene copies are not maintained by selection, one of the gene sequences will eventually mutate to the point at which it does not produce a product, or produces a non-functional product. For example, there are only four functional copies of *hsp70* genes in *D. mauritiana* in contrast to five in *D. melanogaster*. This extra copy might allow *D. melanogaster* to tolerate more extreme environments if having an additional copy allows them to survive in a wider range of temperatures (Bettencourt and Feder 2001).

*Drosophila mauritiana* and *D. sechellia* have an increased sensitivity to heat and cold stress compared to both *D. melanogaster* and *D. simulans* (Stanley *et al.* 1980; Hoffmann *et al.* 2003). *D. mauritiana* is less tolerant to both heat and cold stresses compared to both *D. melanogaster* and *D. simulans* at the adult stage (Stanley *et al.* 1980; Hoffmann *et al.* 2003). The survival of *D. mauritiana* adults was also found to be significantly lower compared to individuals of the other three species after a cold pre-treatment acclimation (Nyamukondiwa *et al.* 2011). At the larval stage, both *D. mauritiana* and *D. sechellia* are also less tolerant to heat stresses than *D. melanogaster* and *D. simulans* (Nyamukondiwa *et al.* 2011). In one study, *D. sechellia* was the least cold tolerant species at the larval stage out of 22 species tested (Strachan *et al.* 2012). The sexes also appear to be affected differently from one another; higher temperatures disproportionately affected male *D. mauritiana* over females, with a significant drop in overall fertility at the adult stage (Matute *et al.* 2009). In general, the island endemics seem to have a reduced tolerance to extreme temperatures compared to the more widespread species. However, the thermal biology of both of these species has not yet been tested across all of their life stages.

#### 1.3.1.5 Thermal biology of *D. nepalensis*

*Drosophila nepalensis* is a species that is outside of the *D. melanogaster* species subgroup but is still within the *D. melanogaster* group. *D. nepalensis* is restricted to the highlands of the Himalaya Mountains of India and Nepal. Originally discovered by Okada in 1954 in the foothills of the mountains in Nepal, the species has recently undergone a range contraction into the highlands, presumably in response to changes in climate (Rajpurohit *et al.* 2008; Parkash *et al.* 2013). The range contraction suggests that *D. nepalensis* is cold adapted. This hypothesis is supported by a laboratory study that found that *D. nepalensis* shows a significant decrease in fitness above 25 °C (Singh 2012). Genetic differentiation among populations is very low for thermal tolerance tested from different areas of the Himalaya Mountains (Singh 2012), indicating that this species may have a reduced ability to respond to a new thermal environment. To persist in a warming climate, therefore, *D. nepalensis* must move to higher altitudes to find suitable temperatures (Parkash *et al.* 2013).

## 1.4 Statement of purpose

While past studies have investigated thermal adaptation in the *Drosophila melanogaster* species group, many were limited because they did not examine these species across their entire geographic range or did not measure life history traits across the entire life cycle. Additionally, many previous studies focused on the tolerance of populations to extreme temperatures rather than examining their fitness at intermediate temperatures. However, with predicted climate warming, organisms will first be exposed to moderate increases in temperature and their ability to adapt to increases in temperature will determine their fitness and survival. The *D. melanogaster* group serves as an ideal model for comparing close relatives that are widespread to those that have small geographic ranges. By comparing the response that we see within and between the widespread or restricted groups, we can make generalizations about how other species will respond to climate change. Moreover, understanding the fitness response to moderate increases in temperature might provide important insight into the adaptive constraints that will potentially be reached with climate change.

As documented in the following chapter (Chapter 2), my objective was to determine whether a widespread species has adapted to the thermal environment across its entire range as well as to determine whether certain life stages are affected by changes in temperature more than others. To investigate this objective, I used populations of a cosmopolitan species, *D. melanogaster*, from five continents and from both continental and island locations. I measured life history traits of the egg, larval, pupal, and adult life stages across a range of temperatures that populations might experience in their native environment. Since *D. melanogaster* rapidly forms geographic clines for thermal tolerance within its native environment, I predicted that populations will be locally adapted to their native environmental temperatures across their entire life cycle. In Chapter 3, my objectives were to study how species with small, geographically restricted ranges will tolerate shifts in temperatures, to see if there are differences among species in thermal tolerance, and to determine whether certain life stages are more sensitive to temperature shifts in these species. I repeated the experiments from Chapter 2, but used three species of the *D. melanogaster* species group with geographically small-sized

ranges: *D. mauritiana*, *D. sechellia*, and *D. nepalensis*. I predicted that since these populations have been geographically isolated with very different climatic conditions for hundreds of thousands of years, these species are genetically differentiated and adapted to their native temperatures across their entire life cycle. In Chapter 4 I compared the results of the study of the widespread species, *D. melanogaster*, with those species with geographically restricted ranges to examine how these species adapt to their thermal environment. Results from these studies may help us understand how organisms will respond to changes in temperature in their native environments.

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# Chapter 2

# 2 Local thermal adaptation detected during multiple life stages in eleven populations of *D. melanogaster* from five continents

## 2.1 Introduction

Adaptation is a heritable response of organisms to their environment. Adaptations arise through natural selection and result in an increase in fitness (Kawecki and Ebert 2004). One way in which organisms can evolve in response to their environment is through local adaptation, which occurs when populations become genetically specialized to their unique environment over generational time. To become locally adapted to an environment, populations evolve traits or trait values that provide an advantage under local conditions over populations from other locations that do not experience the same conditions (Kawecki and Ebert 2004). Alternatively, populations may persist in their environment via phenotypic plasticity, which allows organisms to adjust their phenotype to the local conditions they experience (Angilletta 2009). These two modes of adaptation represent extreme ends of a spectrum. In reality, organisms might use a combination of these two modes of adaptation to persist in their native environments. If climate is predictable and consistent, populations may benefit by specializing to their thermal environment through local adaptation (Kawecki and Ebert 2004; Angilletta 2009). In contrast, if climate is less predictable, it may be beneficial for individuals to use phenotypic plasticity to adjust during development to a particular thermal environment (Angilletta 2009). However, there may be limitations on which mode is used since different life stages might be more phenotypically plastic compared to others, and because both phenotypic plasticity and local adaptation come with potential costs. Individuals may suffer fitness consequences if the environment changes in locally-adapted populations, or if individuals adjust incorrectly through phenotypic plasticity.

Although adaptation is very important for the survival of a population, the process of adaptation is difficult to measure directly. To detect local adaptation, the fitness of resident populations must be greater, on average, when compared to the fitness of non-resident populations in the native environment of the resident population (Kawecki and Ebert 2004; Sinclair *et al.* 2012). Studies that examine local adaptation have tested for significant genotype by environment interactions for traits related to fitness (Kawecki and Ebert 2004). If there is a significant interaction then the researcher examines the interaction to determine if the residents are more fit compared to non-residents in their local conditions. Only if both criteria are met is it possible to infer that local adaptation is occurring. Otherwise, genetic drift might account for the variation among strains (Kawecki and Ebert 2004). In contrast, if individuals are able to survive equally well in a variety of environments, then phenotypic plasticity or a wide tolerance to many different environments might be responsible for their survival.

Local adaptation to thermal environments has been detected in ectotherms, whose body temperatures closely follow the ambient temperature (Sinclair *et al.* 2012; Kawecki and Ebert 2004). If the ambient temperature exceeds the upper limit of temperatures that ectotherms can physiologically tolerate, the organism might not survive (Angilletta 2009). Therefore, the range of temperatures that ectotherms experience in their environment can affect their fitness (Addo-Bediako *et al.* 2000; Angilletta 2009). The ability to survive shifts in temperature is increasingly important in the face of ongoing and rapid climate change (Gaston *et al.* 2009; Chown *et al.* 2010). Populations of organisms that are locally specialized to particular climatic conditions will be forced to change rapidly to adjust to new environmental conditions, or change their habitat range to reflect their thermal tolerance (Chown *et al.* 2010). Organisms that are unable to tolerate changes in temperature might face extirpation or even extinction (Parkash *et al.* 2013). Local thermal adaptation was detected along an altitudinal gradient in an Argentinian population of a fruit fly species, *Drosophila buzzatii* (Sørensen *et al.* 2005). A close relative, *D. melanogaster* shows heat and cold resistance clines, which might indicate local adaptation in this species for these traits (Hoffmann *et al.* 2002). In contrast, those individuals that exhibit phenotypic plasticity can potentially adjust their development to cope with changes in temperature. Many studies focus on the tolerance of populations to

extreme temperatures rather than examining their fitness at an intermediate range of temperatures. However, with climate change, organisms will first be exposed to small changes in temperature and their ability to adapt to these initial shifts will determine their fitness and survival (Prince and Parsons 1977; Mount 1979; Angilletta *et al.* 2010; but see Dillon *et al.* 2009). Our current understanding of the fitness response to these small temperature shifts is limited, and further study will allow us to determine if there will be limits to adaptation with climate change.

The rapid developmental time of *Drosophila* is useful for studying life history traits in the laboratory (Demerec 1950). *D. melanogaster* and its closest relatives originated in Africa and then successfully migrated around the globe, except for the high arctic and Antarctica (David and Capy 1988; Markow and O'Grady 2005). Two of the most widely-studied species, *D. simulans* and *D. melanogaster*, are found worldwide (Sturtevant 1920). A number of studies have used *Drosophila* to examine their survival, reproduction, and physiological response to changing temperatures by using samples from one or two geographical regions, or by comparing single strains of two *Drosophila* species (Tantawy and Mallah 1961; Giesel *et al.* 1982; Montchamp-Moreau 1983; David *et al.* 2004). More comprehensive studies have measured adaptation across a single continental cline (Ayrinhac *et al.* 2004; Sørensen *et al.* 2005; Hoffmann and Weeks 2007; Calabria *et al.* 2012), but few have looked at the divergence of a species across its entire range (Capy *et al.* 1993; Hoffmann *et al.* 2002; Austin and Moehring 2013).

Comparative studies have measured genetic and physiological differences related to survival in species of the *D. melanogaster* subgroup (David *et al.* 2004; Tamura *et al.* 2004; Cutter 2008). Overall, *D. melanogaster* has greater genetic differentiation and more variation in morphological traits among populations compared to its close relative, *D. simulans* (Chippindale *et al.* 1997; Irvin *et al.* 1998; Chakir *et al.* 2002; Capy and Gibert 2004; David *et al.* 2004). This variation suggests that *D. melanogaster* is more locally adapted to its environment, whereas *D. simulans* populations have not genetically differentiated from one another and are thought to be phenotypically plastic for thermal tolerance (Capy and Gibert 2004; Gibert *et al.* 2004; Trotta *et al.* 2006). These two closely-related species may therefore be an excellent model for comparisons of how

phenotypically-plastic and locally-adapted species will respond to changes in temperature. Previous work on the optimal temperature range of *D. simulans* supports the assumption that there is a wide range of temperatures within which this species can perform at an optimal level (Austin and Moehring 2013). Subtle changes in temperature will likely have a minimal effect on *D. simulans* since the species appears to be very phenotypically plastic among populations. However, a comparative study that comprehensively examines the fitness response of many populations across a range of temperatures at different life stages has not yet been completed on *D. melanogaster*.

The effect of temperature on traits other than survival in *D. melanogaster* has been examined through reaction norms, including the effect of temperature on body size (Bakker 1959; Capy *et al*. 1993; Reeve *et al.* 2001; Hoffmann and Weeks 2007), reproductive output (Dillon *et al.* 2007; Marshall and Sinclair 2010), and offspring sex ratios (Tantawy and Mallah 1961; Burke and Little 1995; Pétavy *et al.* 2001; Marshall and Sinclair 2010). Body size affects the mating success and the fecundity of individuals (Anderson 1973; Partridge *et al.* 1994; Hoffmann *et al.* 2001a), while sex ratios can strongly affect population dynamics since the number of females in a population often influences the future population size (Bateman 1948). These thermal reaction norms provide a profile of phenotypes across a range of temperatures and can be used to measure phenotypic plasticity (Via and Lande 1985; Kingsolver *et al.* 2004). Other stresses that affect survival include desiccation, cold, and heat stress. These traits show both local adaptation and phenotypic plasticity among different species of the genus *Drosophila* (Partridge *et al.* 1995; Sørensen *et al.* 2005; Parkash *et al.* 2012). However, it is unclear whether the effects of these stresses are specific to a species as a whole or simply to the small number of populations that were tested.

My objectives were to determine whether a widespread species has adapted to its thermal environment across its entire range and to determine whether certain life stages were more affected by shifts in temperature than others. To this end, I measured thermal adaptation in *D. melanogaster* using populations that had been sampled across the range of the species by subjecting each population to temperatures that span the median annual temperature of their native environment and measuring survival and reproductive traits at

multiple life stages. If the populations of *D. melanogaster* are locally adapted to their native environments, then the thermal reaction norms will vary among populations and the reaction norm peaks will be higher at the native temperature of resident population compared to the reaction norms peaks for non-residents. Since *D. melanogaster* rapidly forms geographic clines for thermal tolerance within its native environment, I predict that populations will be locally adapted to their native environmental temperatures across their entire life cycle.

## 2.2 Materials and methods

### 2.2.1 *Drosophila* stocks and rearing

*D. melanogaster* were collected from ten geographic locations (Table 2.1). These strains were maintained for many generations at the *Drosophila* Species Stock Center at 23 °C until three weeks before experiments began. One additional wild-caught population of *D. melanogaster* was created by pooling 35 isofemale lines that were collected in 2007 from London and Niagara Falls, Ontario (LNF; Marshall and Sinclair 2010). All *Drosophila* stocks were reared on approximately 7 mL of the Bloomington *Drosophila* Stock Center's agar⁄cornmeal⁄yeast-based medium recipe, without malt (Lakovaara 1969), in 30 mL vials ('food vials'), and maintained at 21 °C on a 14 h : 10 h light-dark cycle and 75 ± 10 % relative humidity. Experiments were performed at a range of temperatures chosen to surround the reported optimum temperature for *D. simulans* of approximately 21.3 °C and span the temperatures that strains of *D. melanogaster* experience in their native environment (Table 2.1; Pétavy *et al.* 2001).

### 2.2.2 Egg hatchability

Flies were transferred to population cages containing grape juice and agar-based medium with hydrated active yeast in a Petri dish to allow for egg laying for 16 h at 21 °C. The grape juice medium eased visualization of the eggs. Fifty eggs were transferred to food vials for the experimental temperature treatment (6, 10, 14, 18, 21, 24, 27, 30, 33, and 36 °C; n = four batches of fifty eggs for each strain at each temperature). The number of

**Table 2.1** Origin of *Drosophila melanogaster* strains used for temperature assays sorted by increasing degrees of latitude.

| **Strain Number**[1] | **Origin Location**[2] | **Year Population Sampled**[2] | **Latitude, Longitude** | **Mean of Three Monthly Temperatures (°C)**[4] | |
|---|---|---|---|---|---|
| | | | | **Warmest** | **Coldest** |
| 14021-0231. 24 | Kisangani, Congo | 2003 | 0° 52'N, 25°19'E | 25.1 | 24.2 |
| 14021-0231.123 | Seychelles | 1987 | 4°67'S, 55°49'E | 28.0 | 26.0 |
| 14021-0231.133 | Cusco, Peru | 2009 | 13°51'S, 71°97'W | 13.2 | 10.3 |
| 14021-0231. 134 | American Samoa | 2009 | 13°84'S,171°78'W | 28.2 | 27.0 |
| 14021-0231. 53 | Le Reduit, Mauritius | 2006 | 20°13'S, 57°28'E | 26.0 | 18.7 |
| 14021-0231. 137 | Ogasawara Islands, Japan | 2009 | 27°04'N, 142°12'E | 27.7 | 17.8 |
| 14021-0231. 131 | La Jolla, California | 2009 | 32°88'N, 117°24'W | 20.6 | 14.3 |
| 14021-0231. 51 | Cape Town, South Africa | 2007 | 33°91'S, 18°41'E | 20.7 | 12.3 |
| 14021-0231. 23 | Crete, Greece | 2002 | 35° N, 25°E | 24.5 | 12.2 |
| LNF | London and Niagara-on-the-Lake, Canada | 2007 | 43°80'N, 81°81'W, 43°80'N, 79°80'W | 19.8 | -4.2 |
| 14021-0231. 130 | Queensferry, Scotland | 2009 | 55°97'N, 55°97'W | 13.9 | 4.3 |

[1] Names of strains are referred to by the last three digits (following 14021-0231.).

[2] Data provided by the *Drosophila* Species Stock Center.

[4] Data from the National Oceanic and Atmospheric Administration (NOAA).

eggs that hatched after 96 hours was counted and the larvae were reared at 21 °C to adulthood. My preliminary experiments suggested that no further eggs hatched after 96 h of temperature incubation at any of the experimental temperatures. The sex ratios and mass of males and females were measured for each line and temperature approximately 21 days following initial incubation after the adults had eclosed. Flies were dried overnight before the dry mass of individual flies (n = three flies of each strain and sex at each temperature) was determined using an MX5 microbalance (± 0.5 μg; Mettler Toledo, Columbus, OH, USA).

### 2.2.3 Larval survival & development time

Five adult flies of each sex were placed together in a 30 mL food vial at 21 °C to allow for egg laying. After 24 h, the adults were removed from the vials and the 1$^{st}$ instar larvae were incubated at each experimental temperature (6, 10, 14, 18, 21, 24, 27, 30, 33, and 36 °C; n = four vials for each strain at each temperature). The number of larvae was not standardized among strains because I focused on the differences within a strain among temperatures. Flies that eclosed were removed from the experimental temperatures, counted, and sexed daily to prevent any additional eggs being laid on the food medium. This allowed me to determine the number of eclosing flies and mean development time of each strain, at each experimental temperature. The number of males and females that eclosed from each vial was used to determine the development time and sex ratio. The assays were discontinued when five days passed and no new flies eclosed from the vial, or if no larvae appeared after 60 days. The dry mass of males and females was measured as outlined above.

### 2.2.4 Pupal survival & development time

Five adult flies of each sex were placed together in a 30 mL food vial at 21 °C to allow for egg laying. The number of larvae was not standardized among strains because I focused on the differences within a strain among temperatures. After seven days, the adults were removed and ten wandering-stage larvae were transferred to fresh food vials

and maintained at 21 °C for 24 h to allow development into pupae. Eight vials from each strain were incubated at each temperature (6, 10, 14, 18, 21, 24, 27, 30, 33, and 36 °C; n = eight vials for each strain at each temperature). Adult flies that eclosed were removed, counted, and sexed daily to prevent any additional eggs being laid on the food medium. The number of males and females that eclosed from each vial was recorded daily to determine the development time and sex ratio. The assays were discontinued when five days passed with no new flies eclosing from the vial, or if no flies eclosed after 30 days. The dry mass of males and females was measured as outlined above.

### 2.2.5 Adult fitness: mating behaviour

Eggs and larvae were kept at a constant temperature of 21 °C until eclosion. Newly-eclosed virgin flies were acclimated for five days at each experimental temperature (6, 10, 14, 18, 21, 24, 27, 30, 33, and 36 °C; n = 20 mating assays for each strain at each temperature) then paired with a temperature-treated virgin of the opposite sex in a no-choice mating assay in a 30 mL water-misted vial. Each assay took place at the same temperature to which the flies were acclimated. The mating assay began within 1 h of lights-on and lasted for 45 minutes. The mating behaviour of male and female flies was measured by observing the incidence of courtship and copulation behaviours. The proportion of copulating flies was calculated using flies that first courted. This analysis eliminates confounding statistical bias in copulation occurrence with the presence or absence of courtship, since courtship always precedes copulation during the *Drosophila* mating ritual (Spieth 1974). The dry mass of each sex and strain at each temperature was subsequently determined as outlined above.

### 2.2.6 Walking speed

The walking speed was measured by first incubating ten adult flies of each sex and strain for five days to acclimate to the experimental temperatures (14, 18, 21, 24, 27, and 30 °C). Flies were then aspirated singly without anesthesia to a standard 30 mL vial for the assay. Vials were then tapped down onto the surface of a table to knock the flies down to the food surface and the time to climb 10 cm was measured and averaged over three trials

(n = six flies for each strain and sex at each temperature). Any flies that did not climb 10 cm in 999 s were excluded from the analysis.

### 2.2.7 Activity level

Activity level was measured by first incubating ten adult flies of each sex and strain for five days to acclimate to the experimental temperatures (14, 18, 21, 24, 27, and 30 °C). Flies were then aspirated singly without anesthesia to a standard 30 mL vial. The number of seconds during which the fly was walking inside the vial was measured over a period of 30 s, and afterwards the proportion of time active was calculated (n = six flies for each strain and sex at each temperature).

### 2.2.8 Statistical analysis

All hypotheses were tested at $\alpha = 0.05$. Some points were not shown in graphs if the strain could not survive at that temperature to be tested for their performance. Each life stage was analyzed with a generalized linear model (GLZ) using strains, experimental temperature, the quadratic term for experimental temperature, and their interactions to test for consistent variation among strains in their response to changes in temperature, followed by an analysis of deviance (ANODEV) which shows the results for each of the main effects and the interactions. Experimental temperatures were represented by a linear and quadratic effect of temperature on the response variable to allow for humped responses along a temperature gradient. None of the models constrained the intercept to cross at the origin, allowing for a model that predicted a non-zero level of performance at 0 °C.

If the first regression returned a significant interaction term between strain and experimental temperature, a separate GLZ and ANODEV were conducted as above, with each strain renamed with the mean temperature of the three warmest months as well as the three coldest months, at the closest weather station to the collection site and with both climatic metrics included in each model. These climatic metrics have previously been shown to be the best predictor of fitness in thermal adaptation studies of *Drosophila* (Table 2.1; Feder *et al.* 2000; Hoffmann *et al.* 2002). Interaction effects between either

the linear or quadratic effect of temperature and the cold or warm strain term indicate that the genetic differences for thermal tolerance among strains might reflect local adaptation to temperature for that response variable. The best model for each analysis was selected by starting with the fully-parameterized model and then sequentially dropping non-significant predictor terms until a minimally-adequate model was selected, retaining non-significant main effects when interactions were significant predictors of the response variable (Crawley 2007). Statistics are not reported for non-significant terms dropped from the model because they do not have any associated statistics in the minimally-adequate model.

For egg hatchability and pupal survival, the percent survival of each strain and experimental temperature was compared using a generalized linear model (GLZ) with a binomial error distribution. For the larval stage, the number of eclosing flies of each strain and experimental temperature was compared using a GLZ with a Poisson error distribution. In addition, the larval and pupal development time was compared for each strain and experimental temperature using a GLZ with a Gaussian error distribution. For the adult stage, the proportion of males that courted females and the proportion of copulating flies at each temperature and strain was compared using a GLZ with a binomial error distribution. The walking speed for each temperature, strain, and sex was analyzed using a GLZ as outlined above with a Gaussian error distribution. The activity levels were analyzed in the same manner as walking speed, except the response variable was percent activity. A GLZ was also conducted at each life stage to examine the effect of temperature, sex, strain, and their interactions on the mass of the flies after temperature incubation, with females as the reference variable (i.e. when predicting female survival or performance, females are entered into the model as “0” and males as “1”). A GLZ compared the observed ratio of males and females among experimental temperatures to determine if the sex ratio was dependent on temperature.

## 2.3 Results

### 2.3.1 Egg response to temperature

I measured egg hatchability by incubating of a total of 30 000 eggs and then counting the eggs that hatched (Figure 2.1; Appendix 1). According to the minimally-adequate model, after 96h of temperature incubation the egg hatchability depended the main effects of the linear (GLZ; Table 2.2; $\beta = -3.186$, df = 1, $\chi^2 = 56.51$, $P < 0.001$) and quadratic effects of the experimental temperature ($\beta = -0.006$, df = 1, $\chi^2 = 1469.41$, $P < 0.001$) and the strain of the fly (df = 10, $\chi^2 = 180.82$, $P < 0.001$). After incubation at the egg stage, the adult dry mass of *D. melanogaster* depended on the main effects of the strain of the fly (Table 2.3; $\beta = -0.006$, df = 10, $\chi^2 = 360173$, $P < 0.01$), the linear effect of experimental temperature ($\beta = -1.897$, df = 1, $\chi^2 = 49880$, $P < 0.001$), and the sex of the fly, with females being the heavier sex ($\beta = -109.454$, df = 1, $\chi^2 = 1164961$, $P < 0.001$).

### 2.3.2 Larval response to temperature

I measured the larval survival by incubating of a total of 4 400 adults for egg laying and removing 7 065 offspring from all 440 vials after eclosion (Figure 2.2; Appendix 2). The larval eclosion of *D. melanogaster* depended on the main effects of the linear (GLZ; Table 2.2; df = 1, $\chi^2 = 15.4$, $P < 0.001$) and quadratic effects of experimental temperature (df = 1, $\chi^2 = 5563.7$, $P < 0.001$), and the strain (df = 10, $\chi^2 = 2434.1$, $P < 0.001$). Larval eclosion also depended on the interaction between strain and experimental temperature (df = 10, $\chi^2 = 420.7$, $P < 0.001$), and the interaction between strain and the quadratic effect of experimental temperature (df = 10, $\chi^2 = 132.4$, $P < 0.001$). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, the larval eclosion depended on the main effects of the linear (GLZ; Table 2.2; $\beta = 0.690$, df = 1, $\chi^2 = 15.4$, $P < 0.001$) and quadratic effects of experimental temperature ($\beta = -0.017$, df = 1, $\chi^2 = 5563.7$, $P < 0.001$), and the warmest ($\beta = 0.024$, df = 1, $\chi^2 = 52.3$, $P < 0.001$) and coldest monthly native temperatures ($\beta = -0.035$, df = 1, $\chi^2 = 93.9$, $P < 0.001$). Larval eclosion also depended on the interaction between the coldest monthly temperatures and the experimental temperature ($\beta = 0.0006$, df = 1, $\chi^2 = 6.8$, $P < 0.01$), such that local adaptation might be occurring at the larval stage.

**Table 2.2** A comparison of life history traits among eleven strains of *D. melanogaster* after incubation at different temperatures during development.

| Effect | ***P*** | | | | |
|---|---|---|---|---|---|
| | Egg Hatchability | Larval Eclosion | Larval Development Time | Pupal Survival | Pupal Development Time |
| **Variation Among Strains** | | | | | |
| Experimental Temp* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| (Experimental Temp)$^2$ | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Strain | <0.001 | <0.001 | <0.001 | <0.001 | <0.01 |
| Strain*Experimental Temp | N/A | <0.001 | <0.001 | N/A | N/A |
| Strain*(Experimental Temp)$^2$ | N/A | <0.001 | <0.001 | N/A | N/A |
| **Adaptation to Temp** | | | | | |
| Experimental Temp | N/A | <0.001 | <0.001 | N/A | N/A |
| (Experimental Temp)$^2$ | N/A | <0.001 | <0.001 | N/A | N/A |
| Warm Native Temp | N/A | <0.001 | 0.477 | N/A | N/A |
| Cold Native Temp | N/A | <0.001 | <0.05 | N/A | N/A |
| Warm Native Temp* Experimental Temp | N/A | N/A | <0.01 | N/A | N/A |
| Cold Native Temp* Experimental Temp | N/A | <0.001 | <0.05 | N/A | N/A |
| Warm Native Temp* (Experimental Temp)$^2$ | N/A | N/A | N/A | N/A | N/A |
| Cold Native Temp* (Experimental Temp)$^2$ | N/A | N/A | N/A | N/A | N/A |

* Temp = Temperature

N/A = Term was not included in the minimally-adequate model.

**Table 2.3** A comparison of the effect of temperature incubation on the adult mass of eleven strains of *D. melanogaster* during different life stages.

| Effect | *P* | | | |
|---|---|---|---|---|
| | Egg | Larvae | Pupae | Adult |
| **Variation Among Strains** | | | | |
| Experimental Temp* | <0.001 | <0.001 | 0.889 | <0.001 |
| Strain | <0.01 | <0.001 | <0.001 | <0.001 |
| Sex | <0.001 | <0.001 | <0.001 | <0.001 |
| Strain*Experimental Temp | N/A | <0.001 | <0.01 | <0.01 |
| Sex*Experimental Temp | N/A | N/A | N/A | <0.001 |
| **Adaptation to Temp** | | | | |
| Experimental Temp | N/A | <0.001 | N/A | <0.001 |
| Warm Native Temp | N/A | <0.001 | N/A | <0.001 |
| Cold Native Temp | N/A | <0.001 | N/A | <0.05 |
| Sex | N/A | <0.001 | <0.001 | <0.001 |
| Warm Native Temp* Experimental Temp | N/A | N/A | N/A | <0.05 |
| Cold Native Temp* Experimental Temp | N/A | N/A | N/A | <0.01 |
| Experimental Temp* Sex | N/A | N/A | N/A | <0.001 |

* Temp = Temperature

N/A = Term was not included in the minimally-adequate model.

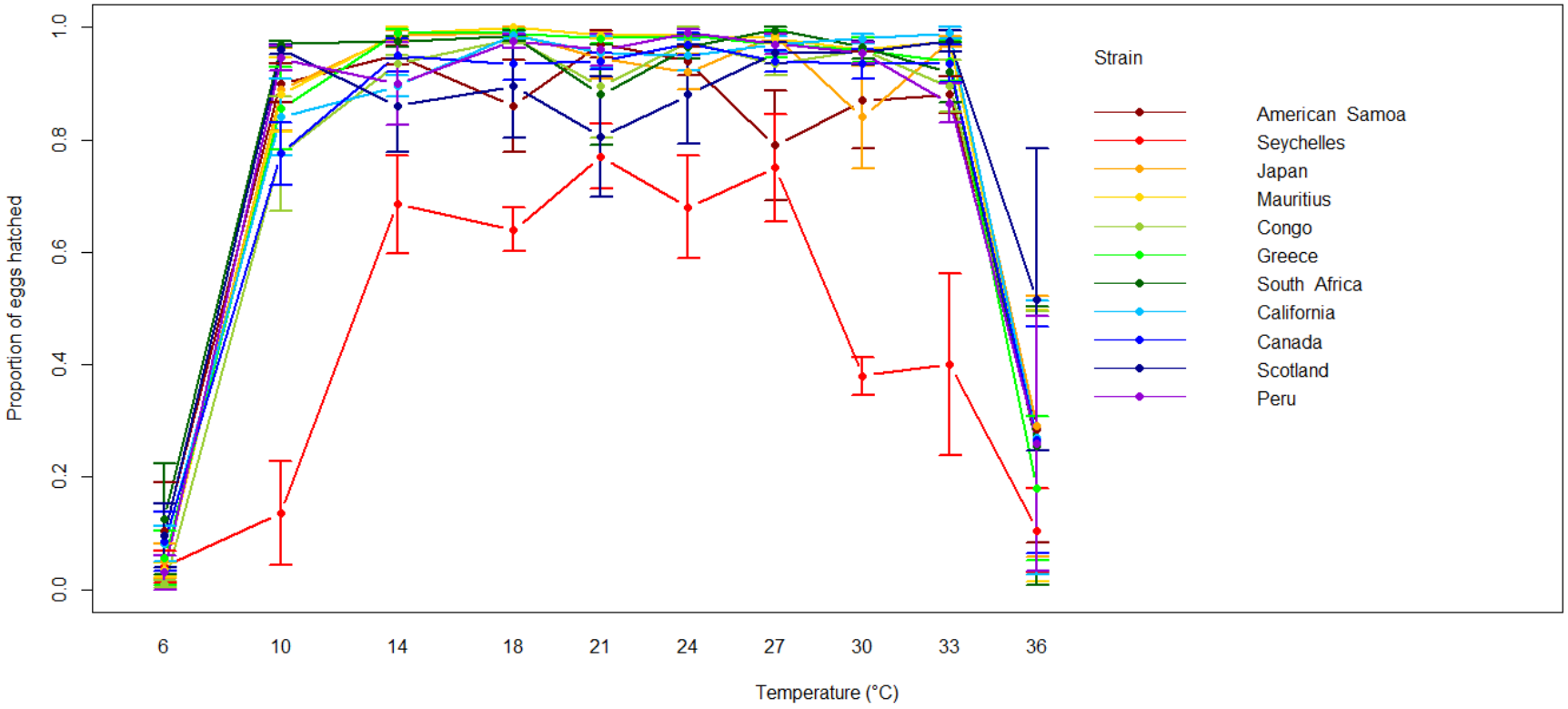


**Figure 2.1** Proportion of eggs hatched at temperatures from 6 – 36 °C for eleven strains of *D. melanogaster* sampled from across its range. Strains are ordered in the legend by the mean of the three warmest monthly temperatures of the original sampling location from the warmest (dark red) to coldest (purple) locations and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

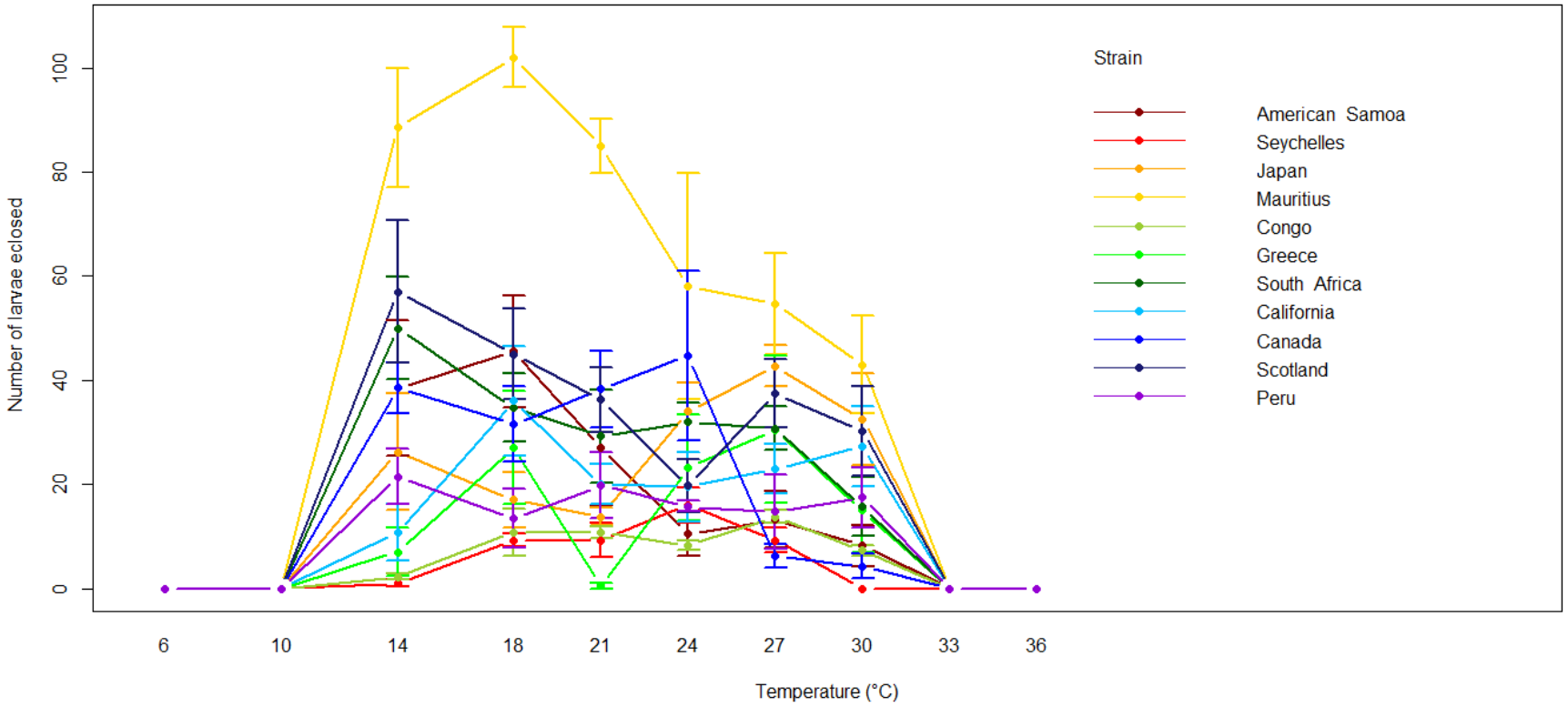


**Figure 2.2** Number of larvae eclosed at temperatures from 6 – 36 °C for eleven strains of *D. melanogaster* sampled from across its range. Strains are ordered in the legend by the mean of the three warmest monthly temperatures of the original sampling location from the warmest (dark red) to coldest (purple) locations and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

The larval development time of *D. melanogaster* depended on the main effects of the linear (GLZ; Table 2.2; df = 1, $\chi^2$ = 13049.9, $P < 0.001$) and quadratic effects of experimental temperature (df = 1, $\chi^2$ = 1961.9, $P < 0.001$), and the strain of the fly (df = 10, $\chi^2$ = 910.3, $P < 0.001$). The interaction between strain and experimental temperature (df = 10, $\chi^2$ = 439.0, $P < 0.001$) and the interaction between strain and the quadratic effect of experimental temperature (df = 10, $\chi^2$ = 156.3, $P < 0.001$; Figure 2.3; Appendix 3) were also statistically significant. After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, the larval development time depended on the main effects of the linear (GLZ; Table 2.2; β = -5.322, df = 1, $\chi^2$ = 13049.9, $P < 0.001$) and quadratic effects of experimental temperature (β = 0.105, df = 1, $\chi^2$ = 1853.5, $P < 0.001$), and the coldest monthly native temperatures (β = -0.421, df = 1, $\chi^2$ = 53.1, $P < 0.05$). The larval development time also depended on the interactions between the warmest (β = -0.039, df = 1, $\chi^2$ = 71.6, $P < 0.01$) and coldest monthly native temperatures and the experimental temperature (β = 0.016, df = 1, $\chi^2$ = 68.4, $P < 0.05$), such that local adaptation might be occurring for the larval life stage. The larval development time did not depend on the main effect of the warmest monthly native temperatures (β = 0.996, df = 1, $\chi^2$ = 5.2, $P = 0.477$).

The adult dry mass of *D. melanogaster* after temperature treatment at the larval stage depended on the main effects of the linear effect of experimental temperature (GLZ; Table 2.3; df = 1, $\chi^2$ = 61440, $P < 0.001$), the strain (df = 10, $\chi^2$ = 97327, $P < 0.001$), and the sex of the fly (df = 1, $\chi^2$ = 262284, $P < 0.001$). The mass also depended on the interaction between strain and experimental temperature (df = 10, $\chi^2$ = 29241, $P < 0.001$). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, there were statistically significant main effects of the linear effect of experimental temperature (β = -2.29, df = 1, $\chi^2$ = 57613, $P < 0.001$), the warmest (β = 0.56, df = 1, $\chi^2$ = 20243, $P < 0.001$) and coldest monthly native temperatures (β = -1.53, df = 1, $\chi^2$ = 33617, $P < 0.001$), and the sex of the fly, with the females being the heavier sex (β = -53.13, df = 1, $\chi^2$ = 261901, $P < 0.001$).

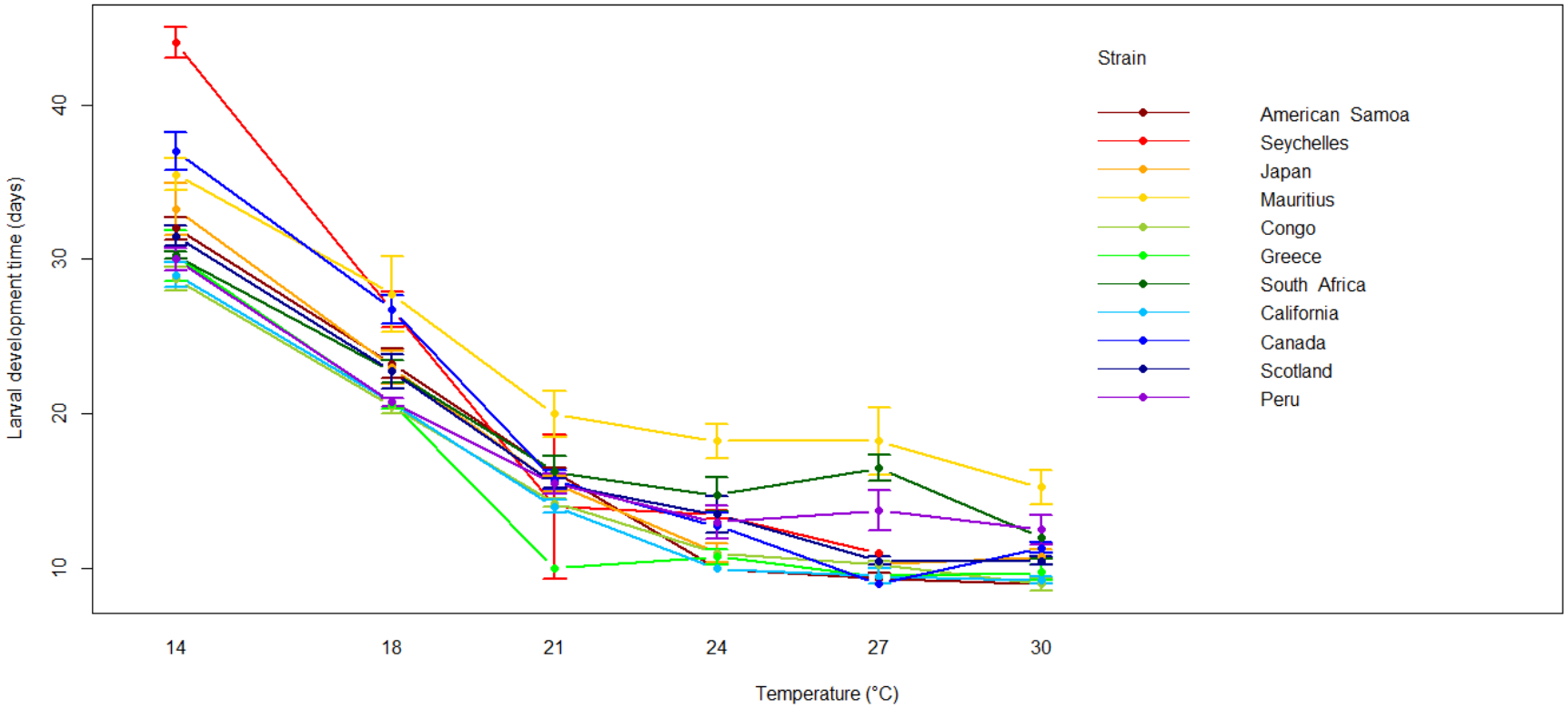


**Figure 2.3** Larval development time at temperatures from 14 – 30 °C for eleven strains of *D. melanogaster* sampled from across its range. Strains are ordered in the legend by the mean of the three warmest monthly temperatures of the original sampling location from the warmest (dark red) to coldest (purple) locations and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

### 2.3.3 Pupal response to temperature

I measured the pupal survival by incubating a total of 8 800 third-instar larvae in 880 vials (Figure 2.4; Table 2.2; Appendix 4). Pupal survival of *D. melanogaster* depended on the main effects of the linear (GLZ; $\beta = 0.819$, df = 1, $\chi^2 = 65.41$, $P < 0.001$) and quadratic effects of experimental temperature ($\beta = -0.019$, df = 1, $\chi^2 = 2211.53$, $P < 0.001$), and the strain of the fly (df = 10, $\chi^2 = 72.45$, $P < 0.001$). Pupal development time depended on the main effects of the linear (GLZ; $\beta = -0.198$, df = 1, $\chi^2 = 3260.1$, $P < 0.001$) and quadratic effects of experimental temperature ($\beta = -53.13$, df = 1, $\chi^2 = 390.6$, $P < 0.001$), and the strain of the fly (df = 1, $\chi^2 = 36.7$, $P < 0.01$; Figure 2.5; Appendix 5).

The mass of *D. melanogaster* after temperature treatment at the pupal stage depended on the strain (Table 2.3; GLZ; df = 10, $\chi^2 = 71687$, $P < 0.001$) and the sex of the fly (df = 1, $\chi^2 = 66395$, $P < 0.001$). The mass also depended on the interaction between strain and experimental temperature (df = 10, $\chi^2 = 32278$, $P < 0.01$). The mass did not depend on the linear effect of experimental temperature (df = 1, $\chi^2 = 25$, $P = 0.889$). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, the minimally-adequate model suggested that the adult dry mass after incubation at the pupal stage depended on the sex of the fly, with females being the heavier sex ($\beta = -51.00$, df = 1, $\chi^2 = 68904$, $P < 0.001$).

### 2.3.4 Adult response to temperature for reproductive behaviours, mass, walking speed, and activity levels

I measured the incidence of courtship and copulation by observing a total of 2 200 pairs of flies (Table 2.4; Figure 2.6; Appendix 6). The courtship incidence of *D. melanogaster* males depended on the linear (GLZ; df = 1, $\chi^2 = 17.38$, $P < 0.001$) and quadratic effects of experimental temperature (df = 1, $\chi^2 = 1734.55$, $P < 0.001$). The male courtship incidence also depended on the interaction between strain and experimental temperature (df = 10, $\chi^2 = 62.44$, $P < 0.001$) and the interaction between strain and the quadratic effect of experimental temperature (df = 10, $\chi^2 = 56.32$, $P < 0.001$). Male courtship incidence

**Table 2.4** A comparison of life history traits among eleven strains of *D. melanogaster* after incubation at different temperatures during the adult life stage.

| Effect | *P* | | | |
|---|---|---|---|---|
| | Courtship | Copulation | Walking Speed | Activity Levels |
| **Variation Among Strains** | | | | |
| Experimental Temp* | <0.001 | <0.001 | <0.001 | <0.001 |
| (Experimental Temp)$^2$ | <0.001 | <0.001 | N/A | N/A |
| Strain | 0.064 | <0.001 | <0.001 | N/A |
| Sex | N/A | N/A | <0.001 | <0.001 |
| Strain*Experimental Temp | <0.001 | <0.001 | <0.001 | N/A |
| Sex*Experimental Temp | <0.001 | <0.001 | <0.001 | <0.05 |
| **Adaptation to Temp** | | | | |
| Experimental Temp | <0.001 | <0.001 | <0.001 | N/A |
| (Experimental Temp)$^2$ | <0.001 | <0.001 | N/A | N/A |
| Warm Native Temp | 0.208 | N/A | <0.001 | N/A |
| Cold Native Temp | <0.001 | N/A | <0.05 | N/A |
| Sex | N/A | N/A | <0.001 | N/A |
| Warm Native Temp* Experimental Temp | N/A | N/A | <0.05 | N/A |
| Cold Native Temp* Experimental Temp | <0.001 | N/A | <0.01 | N/A |
| Warm Native Temp* (Experimental Temp)$^2$ | N/A | N/A | N/A | N/A |
| Cold Native Temp* (Experimental Temp)$^2$ | <0.001 | N/A | N/A | N/A |

* Temp = Temperature

N/A = Term was not included in the minimally-adequate model

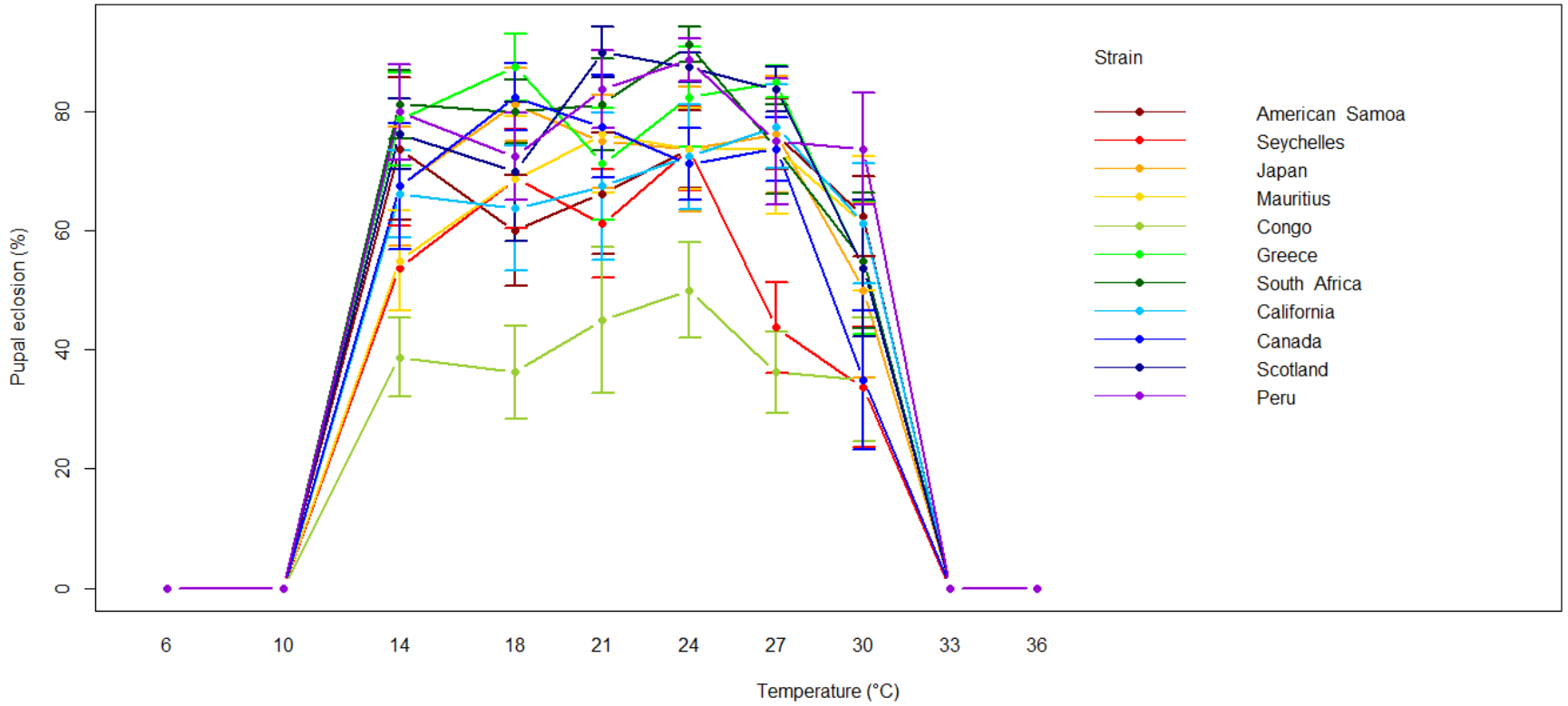


**Figure 2.4** Percent eclosion of pupae at temperatures from 6 – 36 °C for eleven strains of *D. melanogaster* sampled from across its range. Strains are ordered in the legend by the mean of the three warmest monthly temperatures of the original sampling location from the warmest (dark red) to coldest (purple) locations and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

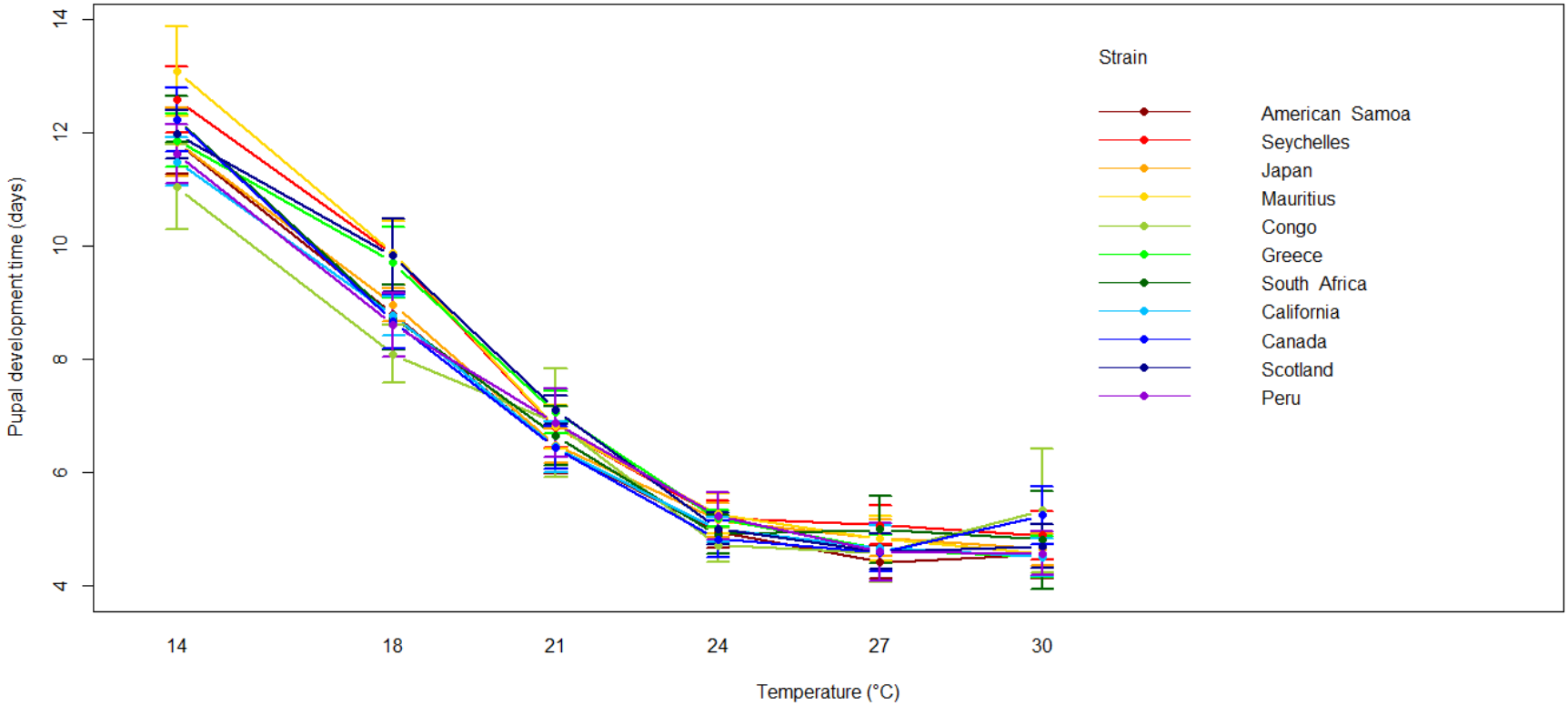


**Figure 2.5** Pupal development time at temperatures from 14 – 30 °C for eleven strains of *D. melanogaster* sampled from across its range. Strains are ordered in the legend by the mean of the three warmest monthly temperatures of the original sampling location from the warmest (dark red) to coldest (purple) locations and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

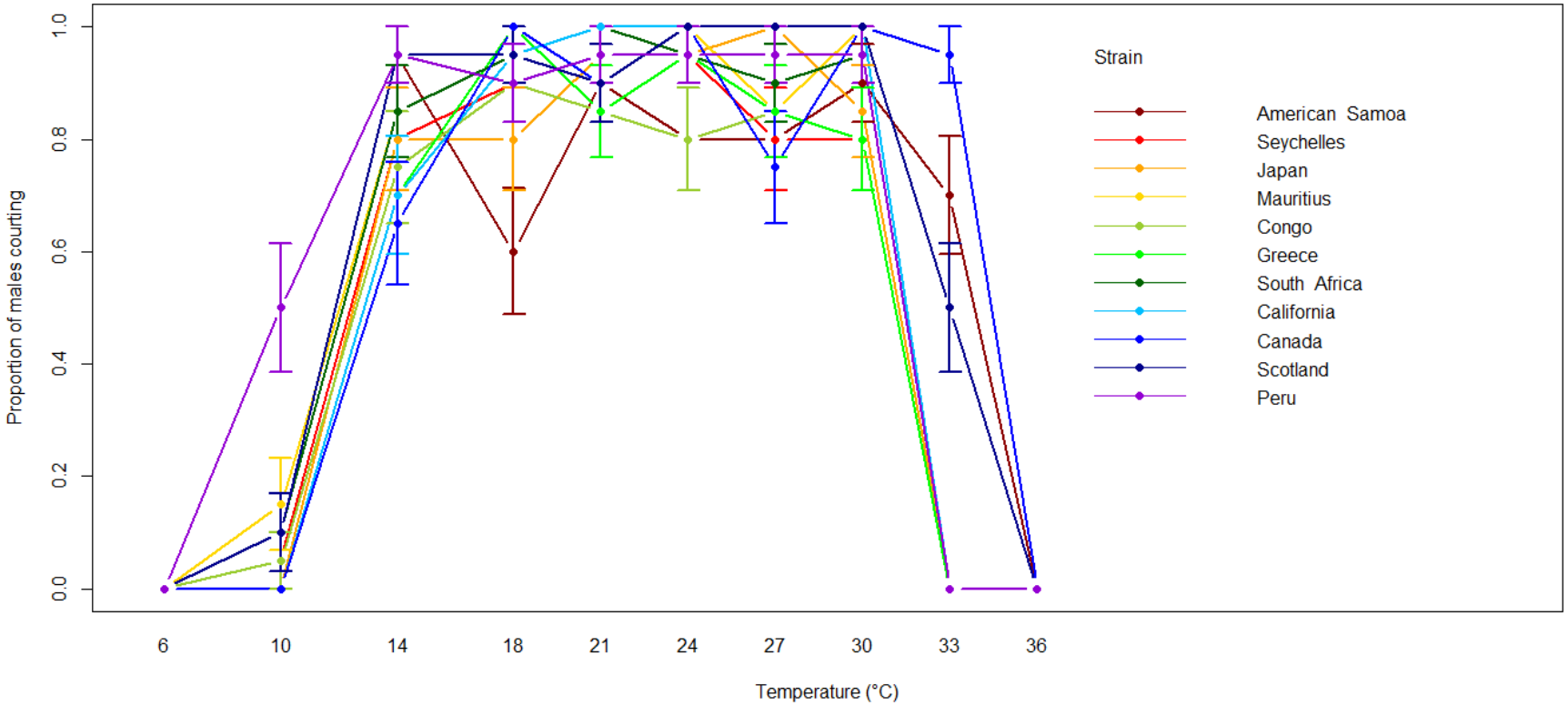


**Figure 2.6** Proportion of males courting during mating assays at temperatures from 6 – 36 °C for eleven strains of *D. melanogaster* sampled from across its range. Strains are ordered in the legend by the mean of the three warmest monthly temperatures of the original sampling location from the warmest (dark red) to coldest (purple) locations and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

did not depend on the main effect of strain (df = 10, $\chi^2$ = 17.49, $P$ = 0.064). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, the male courtship incidence depended on the linear (GLZ; β = 1.849, df = 1, $\chi^2$ = 17.38, $P$ < 0.001) and quadratic effects of experimental temperature (β = -0.042, df = 1, $\chi^2$ = 1515.57, $P$ < 0.001), and the coldest monthly native temperatures (β = -0.098, df = 1, $\chi^2$ = 202.64, $P$ < 0.001). The male courtship incidence also depended on the interaction between the quadratic term for experimental temperatures and the warmest monthly temperatures (β = $8x10^{-5}$, df = 1, $\chi^2$ = 7.87, $P$ < 0.01) and the interaction between the experimental temperatures and the coldest monthly temperatures (β = -0.006, df = 1, $\chi^2$ = 14.93, $P$ < 0.001), such that local adaptation might be occurring at the adult life stage. Male courtship incidence did not depend on the main effect of the warmest monthly native temperatures (β = -0.046, df = 1, $\chi^2$ = 1.58, $P$ = 0.208).

The copulation incidence of *D. melanogaster* depended on the linear (GLZ; Figure 2.7; Table 2.4; Appendix 7; df = 1, $\chi^2$ = 173.877, $P$ < 0.001) and quadratic effects of experimental temperature (df = 1, $\chi^2$ = 205.492, $P$ < 0.001), and the strain of the fly (df = 10, $\chi^2$ = 61.964, $P$ < 0.001). Copulation incidence also depended on the interaction between strain and experimental temperature (df = 10, $\chi^2$ = 33.065, $P$ < 0.001) and the interaction between strain and the quadratic term for experimental temperature (df = 10, $\chi^2$ = 43.500, $P$ < 0.001). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, the copulation incidence depended on the main effects of the linear (GLZ; β = 1.664, df = 1, $\chi^2$ = 173.88, $P$ < 0.001) and quadratic effects of experimental temperature (β = -0.034, df = 1, $\chi^2$ = 223.28, $P$ < 0.001).

The adult dry mass of *D. melanogaster* after temperature treatment at the adult stage depended on the linear term for experimental temperature (Table 2.3; GLZ; df = 1, $\chi^2$ = 76332, $P$ < 0.001), the strain (df = 10, $\chi^2$ = 223101, $P$ < 0.001), and the sex of the fly (df = 1, $\chi^2$ = 1043028, $P$ < 0.001). The mass also depended on the interaction between strain and experimental temperature (df = 10, $\chi^2$ = 125059, $P$ < 0. 01) and the interaction between sex and linear term for experimental temperature (df = 1, $\chi^2$ = 103989, $P$ <

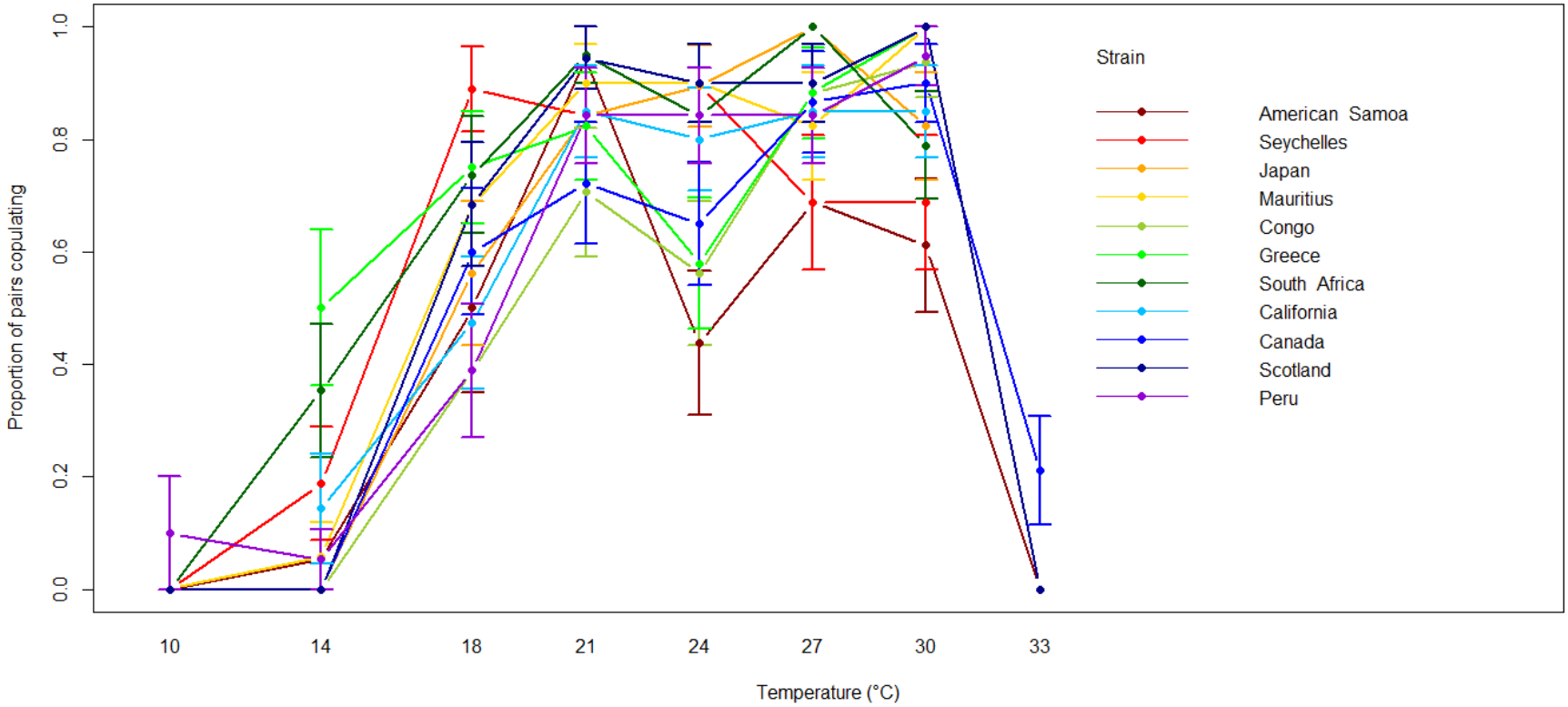


**Figure 2.7** Proportion of pairs copulating during mating assays at temperatures from 6 – 33 °C for eleven strains of *D. melanogaster* sampled from across its range. Strains are ordered in the legend by the mean of the three warmest monthly temperatures of the original sampling location from the warmest (dark red) to coldest (purple) locations and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

0.001). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, the adult dry mass after incubation at the adult stage depended on the main effects of the linear effect of experimental temperature (GLZ; β = 10.468, df = 1, $\chi^2$ = 78272, $P$ < 0.001), the warmest (β = 9.151, df = 1, $\chi^2$ = 86218, $P$ < 0.001) and coldest monthly native temperatures (β = -6.542, df = 1, $\chi^2$ = 22374, $P$ < 0.05), and the sex of the fly (β = -13.048, df = 1, $\chi^2$ = 1042644, $P$ < 0.001). The mass also depended on the interaction between the warmest monthly native temperatures and the experimental temperatures (β = -0.499, df = 1, $\chi^2$ = 26396, $P$ < 0.05), the interaction between the coldest monthly temperatures and the experimental temperatures (β = 0.257, df = 1, $\chi^2$ = 32195, $P$ < 0.01), and the interaction between experimental temperature and the sex of the fly (β = -4.008, df = 1, $\chi^2$ = 104398, $P$ < 0.001).

I measured walking speed by observing a total of 792 individuals (Figure 2.8; Figure 2.9; Table 2.4; Appendix 8; Appendix 9). After five days of temperature incubation the walking speed of *D. melanogaster* depended on the main effects of the linear effect of experimental temperature (β = 0.106, df = 1, $\chi^2$ = 368, $P$ < 0.001) and the sex of the fly (β = -0.414, df = 1, $\chi^2$ = 67.45, $P$ < 0.001). The walking speed also depended on the interaction between the experimental temperature and the sex of the fly (β = -0.045, df = 1, $\chi^2$ = 11.36, $P$ < 0.05).

I measured adult activity level by observing a total of 792 individuals (Figure 2.10; Table 2.4; Appendix 10). After five days of temperature incubation, the activity of *D. melanogaster* depended on the linear term for experimental temperature (GLZ; df = 1, $\chi^2$ = 0.883, $P$ < 0.001), the strain of the fly (df = 10, $\chi^2$ = 1.046, $P$ < 0.001), and the interaction between strain and experimental temperature (df = 1, $\chi^2$ = 0.756, $P$ < 0.01). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, the activity of *D. melanogaster* depended on the linear main effect for experimental temperature (β = -0.027, df = 1, $\chi^2$ = 1.806, $P$ < 0.001) and the interaction between the experimental temperature and the warmest monthly native temperatures (β = 0.001, df = 1, $\chi^2$ = 0.383, $P$ < 0.05), such that local adaptation might be occurring for the adult life stage. The walking speed did not depend on the main effect of the warmest

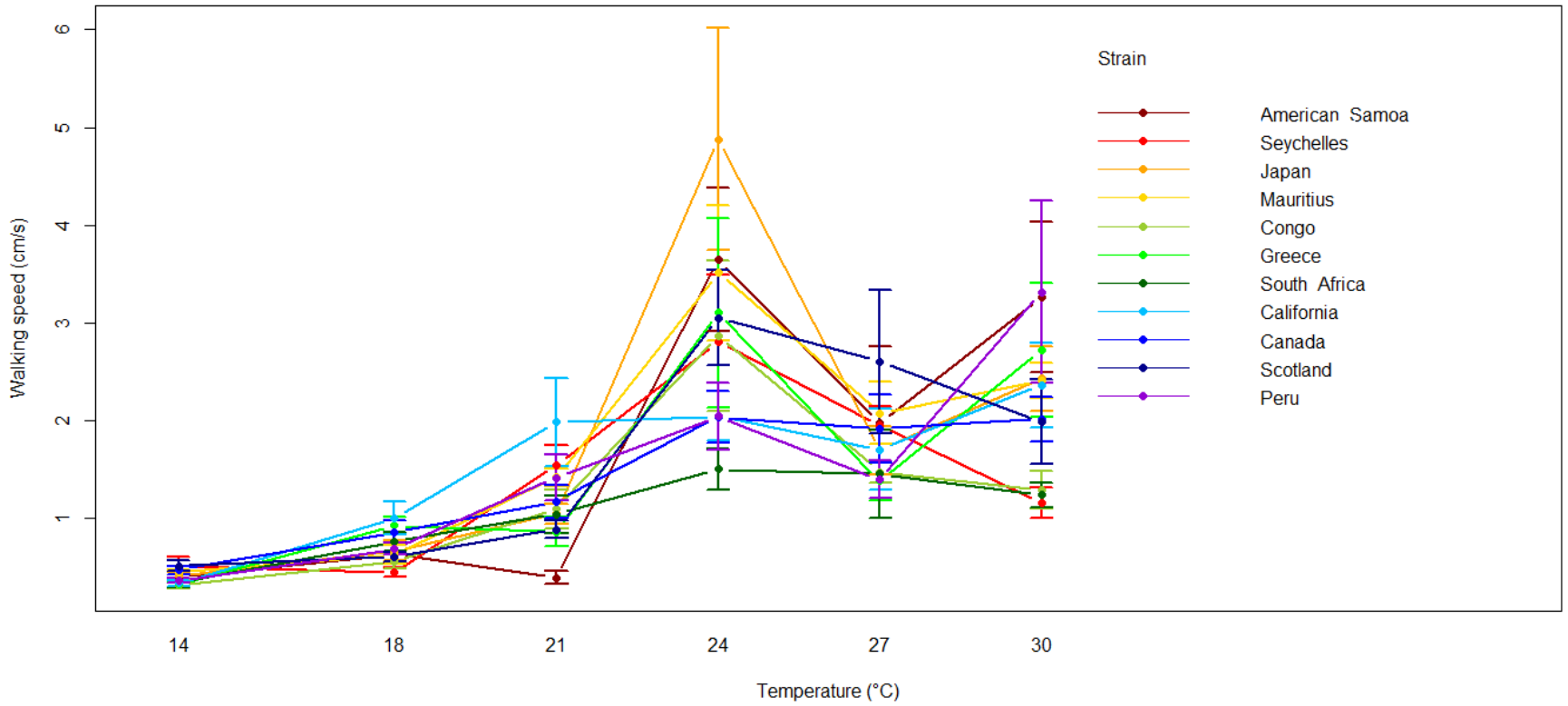


**Figure 2.8** Walking speed at temperatures from 6 – 33 °C for eleven strains of *D. melanogaster* sampled from across its range. Strains are ordered in the legend by the mean of the three warmest monthly temperatures of the original sampling location from the warmest (dark red) to coldest (purple) locations and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

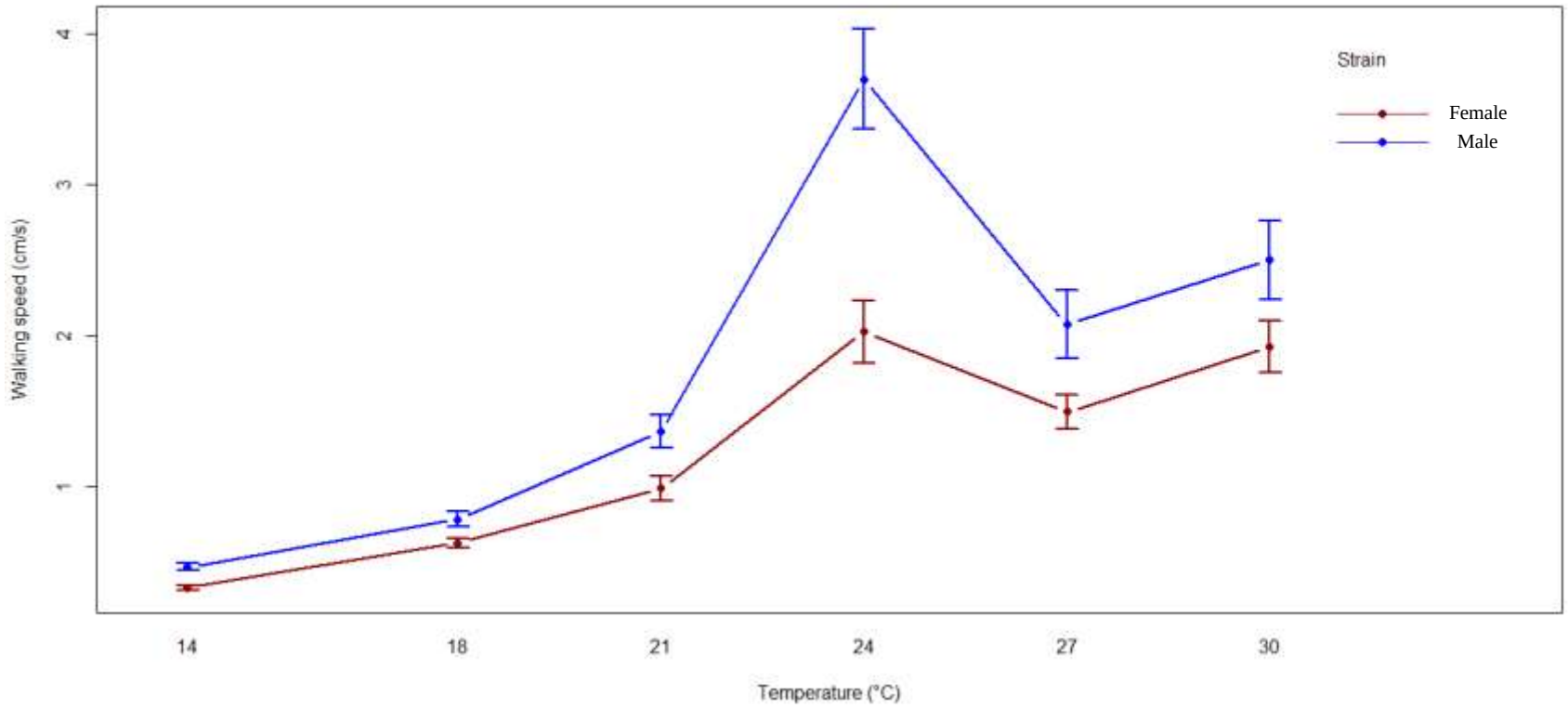


**Figure 2.9** The walking speed at temperatures from 14 – 30 °C, separated by sex, for eleven strains of *D. melanogaster* sampled from across its range. Diamonds represent the speed of a fly walking a distance of 10 cm. Sex is reported at the right of the figure. Each point is the mean value (±SE) for that sex at that temperature.

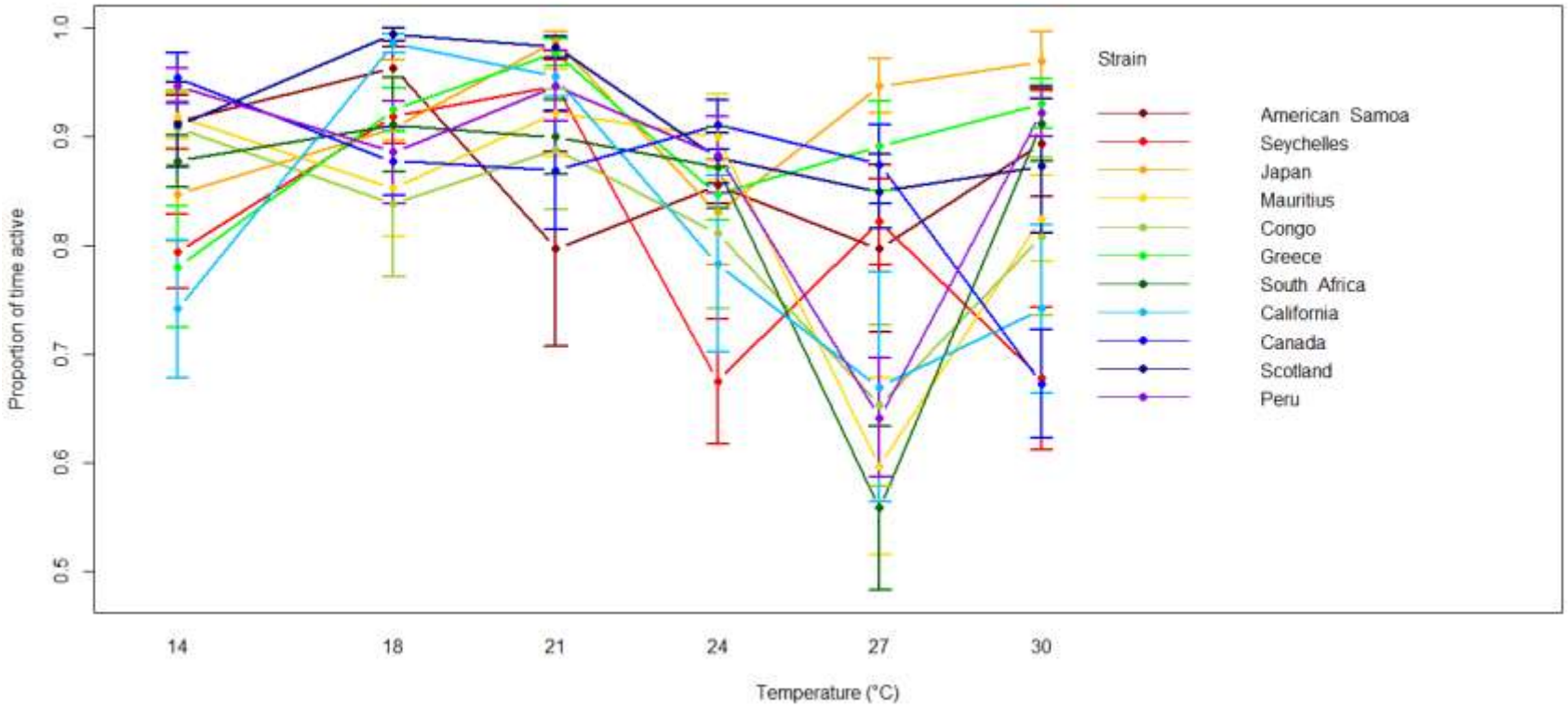


**Figure 2.10** The proportion of time active during 30 s at temperatures from 14 – 30 °C for eleven strains of *D. melanogaster* sampled from across its range. Strains are ordered in the legend by the mean of the three warmest monthly temperatures of the original sampling location from the warmest (dark red) to coldest (purple) locations and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

monthly native temperatures (β = -0.021, df = 1, $\chi^2$ = 0.136, *P* = 0.181).

### 2.3.5 Sex ratios

After incubation at the experimental temperatures, sex ratios were not affected at any of the developmental life stages across the range of temperatures tested (Table 2.5).

## 2.4 Discussion

This study shows that the variation among strains for thermal tolerance is related to native environmental temperatures in *D. melanogaster*, across multiple life stages. Therefore, my data suggest that this species is locally adapted across its entire geographic range. Local adaptation has previously been shown to occur in *D. melanogaster* across clines or small regions (Guerra *et al.* 1997; Nevo *et al.* 1998; Hoffmann *et al.* 2002; Trotta *et al.* 2006; Rashkovetsky *et al.* 2006; Hoffmann and Weeks 2007) and I expand these findings to show local adaptation for the species across its sampling distribution. Across all of its life stages, temperature had an effect on the survival and reproduction of *D. melanogaster*. I detected a significant interaction between strain and experimental temperature in five life history traits related to fitness: larval eclosion, larval development time, male courtship behaviour, copulation behaviours, and activity levels. These interactions represent variation among populations of *D. melanogaster* in response to local adaptation, natural selection, or genetic drift.

A significant strain by environment interaction effect on fitness is required to detect local adaptation among populations (Kawecki and Ebert 2004). At the egg and pupal stages, the survival of *D. melanogaster* depended on the experimental temperature and the strain, but not the native environmental temperature. This suggests that local adaptation is not occurring during these stages (Figure 2.1; Figure 2.4). However, after reanalysis of the data including the coldest and warmest monthly temperatures of the native location of each strain, life history traits at the larval and adult stage had significant interactions between the experimental temperatures and the native temperatures. These interactions in traits directly correlate performance in the laboratory along a range of experimental

**Table 2.5** Generalized linear model (GLZ) for comparison of sex ratios among experimental temperatures during the egg, larval and pupal life stages in *Drosophila melanogaster*.

| **Life Stage** | **Mean %♀** | $\beta^1$ | **df** | $\chi^2$ | ***P*** |
|---|---|---|---|---|---|
| Egg | 50.7 | -0.0002 | 1 | 0.001 | 0.864 |
| Larvae | 49.7 | 0.002 | 1 | 0.023 | 0.357 |
| Pupae | 47.8 | 0.003 | 1 | 0.268 | 0.079 |

temperatures to the native temperatures of these strains, providing evidence that local adaptation to the native environment of each strain is likely occurring for these life history traits.

The traits that were shown to be involved in local adaptation, larval eclosion and development time, male courtship, and adult activity levels are components of the fitness of *D. melanogaster* because they relate to either survival or reproduction. For example, the combined time of larval and pupal development is the longest life stage in *D. melanogaster*, and the relative immobility in these stages compared to the adult life stage makes this period potentially the most vulnerable of all life stages to changes in temperature (Demerec 1950; Dillon *et al.* 2009). Interestingly, local adaptation was detected during the larval but not the pupal life stage. Both larvae and pupae inhabit necrotic fallen fruit, which has been shown to reach internal temperatures of 41 °C in tropical regions (Feder *et al.* 1996). As a result, both stages are exposed to potentially unfavourable temperatures, and yet only the larval stage shows variation among populations for thermal tolerance. This outcome may reflect the complete immobility of the pupal stage, which necessitates tolerance of these potentially lethal temperatures, while the larvae have the ability to move to escape these temperatures.

During the adult life stage, male courtship is affected by interactions between both the coldest and warmest monthly native temperatures, and the experimental temperatures (Figure 2.6). These interactions suggest that the mating behaviour of male *D. melanogaster* is likely to be adapted to both warm and cold temperatures in the native environment of each strain. The incidence of copulation is primarily a measure of the receptiveness of a female to the male courting her, but the surrounding environmental characteristics can also affect her willingness to mate (Spieth 1974; Schnebel and Grossfield 1984). Interestingly, I found a significant interaction between the experimental temperatures and the strain for copulation (Figure 2.7), but after reanalysis, I show that this variation is not related to native temperatures and therefore might reflect differences in the strains due to genetic drift or adaptation to another environmental factor. Additionally, comparing the male mating behaviour trait (courtship) to the female mating

behaviour trait (copulation) shows that male initiation of courtship has a wider range of permissive temperatures than does female receptivity. Activity levels of flies are also affected by the interaction between the warmest monthly native temperatures and the experimental temperature. Activity levels of the flies might therefore be thermally adapted based on the warmest months in the environment from which the strain was sampled (Figure 2.10). In *Drosophila,* walking speed and activity are important measures of performance because they are related to the fitness of that individual (Gibert *et al.* 2001; Dillon *et al.* 2009). For the other life history traits that did not show local adaptation, either phenotypic plasticity or a wide thermal tolerance may be responsible for this species' persistence in each population's native environment.

Although I only used the warmest and coldest monthly temperatures to predict local adaptation in this study, I was able to detect consistent variation among strains related to the native temperatures where the strains were originally sampled. This result suggests that these climate measures are reasonable predictors for the direction of thermal differences among habitats in the native environment of each strain. This result is in agreement with a past study that suggests that the climatic metric that best explains the variation in data is the mean temperature of the warmest and coldest month for high and low temperature measures, respectively (Hoffmann *et al.* 2002). However, an additional analysis using more detailed climate data might have allowed me to detect additional patterns for thermal adaptation in my data, such as annual mean temperature and mean temperature during the time that *Drosophila* are active during the year. These two climatic metrics explained 53% of the variation in models for thermal adaptation that predicted body size in beetles (Stillwell *et al.* 2007). Additionally, the season during which the population was sampled might affect the performance of flies across a range of temperatures (Schmidt, pers. comm.). For instance, if flies were sampled during winter, some less cold-tolerant flies might have entered diapause and would then be excluded from my study, while others that were more cold-tolerant might remain active and could be caught (Schmidt, pers. comm.). These flies might form the representative strain used for that population but might not reflect the mean response of the population to cold. By selecting both the warmest and coldest monthly temperatures, I increased the likelihood of detecting some variation in response to temperature changes that is present in natural

populations (Schmidt, pers. comm.). It is possible that I could not detect some of the variation in thermal tolerance due to a population bottleneck, seasonal differences in genotypes, or a different climate metric. However, I did find consistent genetic variation among strains across the larval and adult life stages that might reflect thermal adaptation. To perform a more accurate study, I would have to collect a very large number of flies from each population over the course of different seasons. These flies could be pooled to create a laboratory population that is representative of natural populations.

Local adaptation to native temperatures was detected in multiple life stages across five continents in *D. melanogaster*, which is in agreement with past literature (reviewed in David *et al.* 2004). Past studies that compare populations along clines of *D. melanogaster* found local adaptation across a smaller geographic scale (Hoffmann *et al.* 2002) and also found local adaptation across larger geographic areas within one or two continents (Guerra *et al.* 1997; Nevo *et al.* 1998; Trotta *et al.* 2006; Rashkovetsky *et al.* 2006; Hoffmann and Weeks 2007). When the thermal biology of *D. melanogaster* was examined on a much wider scale, phenotypic plasticity was determined to be far more important than local adaptation (Ayrinhac *et al.* 2004). In contrast, my study shows that the climate of the original sampling location for each strain does explain most of the variation in survival and reproduction at particular life stages. This difference in results might be because Ayrinhac *et al.* (2004) examined recovery time to cold shock, whereas I looked at survival and reproduction across a range of intermediate temperatures. It therefore appears that *D. melanogaster* has locally adapted to the median native temperatures, but responds plastically to extreme conditions such as cold shock.

A close relative of *D. melanogaster*, *D. simulans*, is also a cosmopolitan species and many studies have compared their thermal biology (reviewed in David *et al.* 2004). In general, *D. melanogaster* is considered to be more thermally adapted and genetically differentiated into populations than *D. simulans* (David *et al*. 2004), and is thought to have a wider range of temperatures that the species can tolerate (Mckenzie 1978; Capy *et al.* 1993; Schnebel and Grossfield 1984). I recently showed that there was a wide tolerance to temperature in *D. simulans* (Austin and Moehring 2013). I now demonstrate that *D. melanogaster* has the same insensitivity to a range of temperatures at the egg

stage as was found in *D. simulans*, with many of the strains having identical levels of egg hatchability from 10–30 °C. At the larval stage, many of the peaks in number of eclosing larvae in *D. melanogaster* are equally as pronounced as those found in *D. simulans*, the difference being that these peaks in *D. melanogaster* are related to temperatures they experience in the wild, which was not the case reported for *D. simulans* (Austin and Moehring 2013). I performed an additional analysis of the data from Austin and Moehring (2013) that included climatic factors in a single model instead of a two-step model using an ANOVA and a correlation to detect local adaptation. This reanalysis confirmed the results of the past study that populations of *D. simulans* are largely not locally adapted to their native temperatures. However, this additional analysis indicated that there might be local adaptation to native temperatures at the larval stage of *D. simulans* (Appendix 11; Kawecki and Ebert 2004). The development time of *D. melanogaster* is slightly faster than the development time of *D. simulans* when measured at lower temperatures, but both of their development times plateau at approximately 10 days at warmer temperatures (Austin and Moehring 2013). The incidence of courtship appears to occur over a wider range of temperatures in *D. melanogaster* compared to courtship in *D. simulans*, but copulation behaviour is observed across a similar range of temperatures in both species (Austin and Moehring 2013), which is consistent with the results of a study by Schnebel and Grossfield (1984). One caveat to comparing the results of the *D. melanogaster* study to the *D. simulans* study for the adult mating behaviour assay is that the current study examined flies at the acclimated temperature, whereas Austin and Moehring (2013) observed flies under common conditions (21 °C) following an acclimation treatment. Nevertheless, the mating behaviours of *D. simulans* males and females appear to have a similar acceptable range of temperatures, while *D. melanogaster* males have an increased range of acceptable temperatures compared to females.

Across the developmental life stages, females were the heavier sex, consistent with past studies looking at the mass of flies (Nunney and Cheung 1997). These sex differences in mass are explained by the direct relationship between mass and fecundity in females (Anderson 1973; David *et al.* 2004). The mass of *D. melanogaster* at the adult stage is affected by many complex interactions between sex, strain, and experimental temperatures, which makes it difficult to draw direct conclusions about differences

among strains and experimental temperatures. Temperature might affect the amount of food consumed, the efficiency of assimilation, growth rates, or the allocation of nutrients to body tissues in *Drosophila* (French *et al.* 1998). Since flies do not show any indication of thermal adaptation to mass during their initial developmental stages but do show thermal adaptation at the adult stage, the mass must be affected at different life stages depending on the native environment of the fly. This result is surprising when compared to the results of the study on *D. simulans*, where I followed identical methods for fly rearing but found no differences among experimental temperatures (Austin and Moehring 2013), indicating that *D. melanogaster* might use their food resources differently in their native environment. The effect of temperature on body size may be influenced by behaviour, such as differential rates of food consumption or through how mass is accumulated within the body. French *et al.* (1998) showed a cumulative effect of rearing temperature on the body and cell size in *D. melanogaster*, with early life stages having the greatest effect on body and cell size and later ones having less of an effect. In contrast, I showed that rearing temperature affected body mass at all life stages, which may be because I tested each life stage individually rather than cumulatively.

The current study of *D. melanogaster* and the previous study of *D. simulans* both found no differences in the sex ratios across all life stages and experimental temperatures (Austin and Moehring 2013). This result is in contrast to what was previously reported (Tantawy and Mallah 1961). My result suggests that temperature is not inducing meiotic drive. However, in this study the exposure to intermediate temperatures was always post-embryonic and therefore temperature might have an effect on sex ratios at the adult stage during gametogenesis. In Tantawy and Mallah (1961) the temperature treatment started before adult reproduction, which might be the stage at which sex ratios are affected by temperature.

Although I detected differences in survival among strains of *D. melanogaster* after temperature incubation, most strains performed similarly across the range of temperatures I tested. One exception is the strain from the Seychelles, which had a lower overall survival compared to the remaining strains during some of the developmental assays. This overall reduction in survival could be due to loss of thermal tolerance due to the

relatively constant temperature in the native environment (mean monthly temperatures only vary *c.* ± 2 °C annually) or could have resulted from inbreeding depression after long-term laboratory maintenance (since 1987; Table 2.1). The majority of the remaining strains were collected from their original location within four years of the start of the experiment (Table 2.1). Some laboratory adaptation might have occurred during this time, as has been demonstrated in past studies of *D. melanogaster* for thermal tolerance and other stresses such as desiccation or starvation resistance (Cavicchi *et al.* 1995; Partridge *et al.* 1995; Krebs and Feder 1997; Hoffmann *et al.* 2001b). However, laboratory stocks of *Drosophila* have been shown to maintain their ability to respond to temperatures that they do not experience in the laboratory (Krebs *et al.* 2001; Nyamukondiwa *et al.* 2011; Strachan *et al.* 2011; Austin and Moehring 2013). Here, I used *D. melanogaster* from different genetic backgrounds and still detected interactions between native conditions and experimental conditions; these thermal optima do not match the maintenance temperature at the *Drosophila* Species Stock Center. Therefore, there is some variation remaining among strains in response to temperature, which might indicate thermal adaptation to the native environment of each strain.

By investigating differences across the life cycle of *D. melanogaster* I have uncovered differences in the ways that flies can tolerate changes in their environment. Certain life stages are better able to tolerate warmer or cooler temperatures than others, which might provide clues as to how these flies live in their native environment. The egg stage, albeit short lived, is very tolerant to warm and cool temperatures, with many strains having equal levels of egg hatchability across a 20 °C range of temperatures; because of this tolerance, females can lay eggs in different environments that might experience more extreme temperatures than larvae or pupae can tolerate. There also appears to be local adaptation at the larval and adult life stages of *D. melanogaster*. While flies may be better adapted to their current environment if they are locally specialized, this specialization may reduce the ability of flies to tolerate changes in the environment compared to more phenotypically plastic species, such as *D. simulans* (David *et al.* 2004; Austin and Moehring 2013). This may place *D. melanogaster* at a greater risk compared to *D. simulans* for extirpation of populations with climate change if each population cannot tolerate changes in temperature. The differences in thermal adaptation that I identified

across an intermediate range of temperatures can tell us which species, both in the *D. melanogaster* species subgroup and in general, are sensitive to changes in their native temperatures and can be used with climate change models to determine susceptibility of species to ongoing climate change.

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# Chapter 3

# 3 Response to temperature shifts by geographically-restricted species within the *D. melanogaster* species group

## 3.1 Introduction

It is increasingly important for us to understand the way that organisms respond to changes in temperature in their environment with ongoing climate change, where many habitats are predicted to change rapidly over time (Angilletta 2009). To study the susceptibility of species and populations to changes in temperature, life history traits related to fitness, including juvenile development time, fertility, and fecundity, can be compared across a range of temperatures (Kawecki and Ebert 2004; Trotta *et al.* 2006). This profile of phenotypes across a range of temperatures is known as a thermal reaction norm and can be compared across species and populations (Via and Lande 1985; Hoffmann and Weeks 2007). Differences in the shape of reaction norms suggest that species or populations respond differently to temperature, either through local specialization or through phenotypic plasticity. Local specialization suggests adaptation to local conditions, whereas phenotypic plasticity allows for individuals to adjust their phenotype to the local conditions that they experience (David *et al.* 2004; Kawecki and Ebert 2004; Angilletta 2009). These two modes are not mutually exclusive and different organisms likely use them in varying combinations to persist in their native environments. If the populations are genetically differentiated then they might not be able to tolerate new temperatures and might face extinction. In contrast, if individuals are more insensitive to temperature or can exhibit phenotypic plasticity, they might be able to tolerate a shift in temperature in their environment (Angilletta 2009).

The way that taxa respond to different temperatures in their environment has been investigated within the *Drosophila melanogaster* species group in multiple ways. Various

methods have been used to examine adaptation including comparing multiple species (Nyamukondiwa *et al.* 2011), comparing two species (Mckenzie 1978; Capy *et al.* 1993; Pétavy *et al.* 2001), and comparing populations within a particular species (Guerra *et al.* 1997; Trotta *et al.* 2006; Hoffmann and Weeks 2007; Austin and Moehring 2013). Certain species, such as *D. melanogaster*, are genetically differentiated for thermal tolerance among populations (Chippindale *et al.* 1997; Irvin *et al.* 1998; Chakir *et al.* 2002; Capy and Gibert 2004; David *et al.* 2004). Others, such as *D. simulans* are less differentiated into populations and are thought to use phenotypic plasticity to survive in different environments (Capy and Gibert 2004; Gibert *et al.* 2004; Trotta *et al.* 2006; Austin and Moehring 2013).

By contrast, species that are restricted to specific habitats, rather than widely distributed habitats like those of *D. melanogaster* and *D. simulans*, might be more sensitive to temperatures that they do not typically experience in their native environment. Populations of the island species *D. mauritiana* and *D. sechellia* exhibit less tolerance to warm and cold stresses compared to the two cosmopolitan species, *D. melanogaster* and *D. simulans* (Stanley *et al*. 1980; Nyamukondiwa *et al.* 2011). The effect of this sensitivity to temperature is further compounded since species with geographically small ranges might face difficulties tracking suitable habitat with ongoing climate change, as climatically suitable habitats might only be present across an oceanic barrier (David *et al.* 2007). The island species *D. mauritiana* and *D. sechellia* are not as tolerant to heat stress as the more cosmopolitan species of *D. melanogaster* and *D. simulans* (Stanley *et al.* 1980; Hoffmann *et al.* 2003; Nyamukondiwa *et al.* 2011); with *D. mauritiana* being more tolerant to heat and cold stress than *D. sechellia* (Table 3.1; Strachan *et al.* 2012). The sexes are not equally affected by this temperature sensitivity, at least in *D. mauritiana*, where the fertility of males is disproportionately affected by heat stress compared to that of females (Matute *et al.* 2009).

A more distantly related species, *D. nepalensis*, is only found in the Himalaya Mountains (Parkash *et al.* 2013). Mountains might act in the same restrictive manner as an island habitat. *D. nepalensis* was recently reported to have undergone range contraction and a decrease in relative abundance over 50 years (Rajpurohit *et al*. 2008; Parkash *et al.*

2013). This range shift is likely in response to warming climate and a lack of phenotypic plasticity, but a comprehensive examination of the thermal tolerance of *D. nepalensis* has not been performed. In general, *D. nepalensis* is a cold-adapted species, having very low survival and fitness above 21 °C (Singh 2012; Parkash *et al.* 2013). A low tolerance to warmer temperatures puts *D. nepalensis* at risk of extinction with climate change.

The effect of changing temperatures has not been well characterized in species with small geographic ranges, especially for multiple life history traits. Collectively, *D. mauritiana, D. sechellia*, and *D. nepalensis* can be used as a model to determine how species with restricted ranges respond to differences in temperatures that are ecologically relevant. Shifts in intermediate temperatures are the first changes that will affect organisms during climate change. If there are genetic differences in the way that species respond to these initial shifts in temperature, they might not be able to tolerate the large changes in temperature predicted by climate change. In contrast, if species are able to survive in many different environments, then they might be able to adjust to changing climate conditions.

My objectives were to study how species with geographically smaller-sized ranges will tolerate a variety of temperatures and to see if there is variation in tolerance among these species. By examining multiple life history traits I determined which life stages were most susceptible to changes in temperature. I measured variation in response to temperature changes in three species with geographically small-sized ranges: *D. mauritiana*, *D. sechellia*, and *D. nepalensis*. I subjected each strain to experimental temperatures similar to the native temperatures each strain would experience in their native environment (Table 3.1) and measured life history traits across their lifespan. If there are genetic differences in the way that these *Drosophila* species respond to temperature, then the shape of thermal reaction norms will vary among them. I predict that since these populations have been geographically isolated with very different climatic conditions for hundreds of thousands of years, these species will be genetically differentiated and adapted to their native temperatures across their entire life cycle.

## 3.2 Materials and methods

Two strains of *Drosophila mauritiana* and one strain each of *D. sechellia* and *D. nepalensis* (Table 3.1) were tested for local adaptation to temperature by examining egg hatchability, larval survival and development time, pupal survival and development time, adult mating behavior, adult walking speed, adult activity, adult mass, and adult sex ratio after incubation at each life stage. During certain experiments *D. nepalensis* and *D. sechellia* were not able to be tested because no flies survived at these temperatures. The methods are identical to those presented in Chapter 2, with the following modifications:

### 3.2.1 *Drosophila* stocks and rearing

One strain of *D. mauritiana* (MauM) was created by pooling five isofemale lines that I collected in 2012 from Île Maurice, Mauritius. Another strain of *D. mauritiana* (MauR) was created by pooling four isofemale strains I collected during the same week from Rodrigues Island, Mauritius. Both strains have since been reared on approximately 7 mL of the standard Bloomington *Drosophila* Stock Center's agar/cornmeal/yeast-based medium without malt (Lakovaara 1969) in 30 mL vials ('food vials') and maintained at 21 °C on a 14 h : 10 h light-dark cycle and 75 ± 10 % relative humidity. Wild-caught *D. sechellia* (SechA) from Anse Royale, Seychelles were sampled by Daniel Matute in 2012, and a synthetic strain made from 10 isofemale lines was provided to the Moehring laboratory in London, ON, Canada in the same year. The strain has since been reared as described above but with the addition of 0.5% v/v octanoic acid, one of the active ingredients in the host plant of the fly that promotes egg laying (Markow *et al.* 2009). During all of the following experiments, *D. sechellia* flies laid eggs, were reared, and were maintained as adults on blue-dyed food with 0.5% v/v octanoic acid. Lastly, a laboratory line of *D. nepalensis* (Nep) was provided to the Moehring laboratory in 2012 (Strachan *et al.* 2011). The strain has since been maintained as described above, except on the Bloomington *Drosophila* Stock Center standard banana-based food medium.

**Table 3.1** Origin of *Drosophila* spp. strains used for temperature assays, sorted by increasing degrees of latitude

| **Strain Name** | **Species** | **Origin Location** | **Year Population Sampled**[1] | **Latitude, Longitude**[1] | **Mean of Three Monthly Temps**[2] **(°C)** | |
|---|---|---|---|---|---|---|
| | | | | | **Warmest** | **Coldest** |
| SechA | *D. sechellia* | Anse Royale, Seychelles | 2012 | 4°7’S, 55°52’E | 28.0 | 26.2 |
| MauR | *D. mauritiana* | Rodrigues, Mauritius | 2012 | 19°69’S, 63°41’E | 26.5 | 22.1 |
| MauM | *D. mauritiana* | Île Maurice, Mauritius | 2012 | 20°13'S, 57°28'E | 26.4 | 21.2 |
| Nep | *D. nepalensis* | Shimla, India | 2003-2004[3] | 31°6′N 77°10′E | 18.3 | 6.2 |

[1] Data from maps.google.com.

[2] Temps = temperatures in °C; Data from the National Oceanic and Atmospheric Administration (NOAA).

[3] The exact year is not known, however, the stock origin is described as recently collected in Parkash *et al.* 2005.

### 3.2.2 Egg hatchability

Flies were transferred to population cages containing blue-dyed cornmeal based medium with hydrated active yeast in a Petri dish to allow for egg laying for 16 h at 21 °C. The blue dye eases visualization of the eggs. *D. nepalensis* laid eggs in very low numbers over the 16 h period, so this assay was not possible given the quick development time of *Drosophila* eggs because by the time enough eggs were laid by *D. nepalensis*, the other eggs of each species had already begun hatching (Markow *et al.* 2009).

### 3.2.3 Pupal survival & development time

Flies were transferred to population cages containing blue-dyed cornmeal-based medium with hydrated active yeast and a small amount of banana medium to the surface of the food in a Petri dish to allow for egg laying. After seven days, the adults were removed and ten wandering-stage larvae were transferred to fresh food vials and maintained at 21 °C for 24 h to allow development into pupae.

## 3.3 Results

### 3.3.1 Egg response to temperature

I measured egg hatchability by incubating of a total of 6 000 eggs over the range of temperatures from 6 – 36 °C (Figure 3.1; Appendix 12). According to the minimally-adequate model, egg hatchability depended on the linear (Table 3.2; GLZ; df = 1, $\chi^2$ = 58.51, $P < 0.001$) and quadratic effects of the experimental temperature (df = 1, $\chi^2$ = 17.38, $P < 0.001$), and the strain of the fly (df = 2, $\chi^2$ = 578.75, $P < 0.001$). Egg hatchability also depended on the interaction between strain and the linear effect of experimental temperature (df = 2, $\chi^2$ = 38.96, $P < 0.001$) and the interaction between the strain of the fly and the quadratic effect of experimental temperature (df = 2, $\chi^2$ = 128.25, $P < 0.001$). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, egg hatchability depended on the main effects of the linear (Table 3.2; GLZ; $\beta$ = 21.190, df = 1, $\chi^2$ = 58.51, $P < 0.001$) and quadratic effects of the experimental temperature ($\beta$ = 0.442, df = 1, $\chi^2$ = 800.03, $P < 0.001$), and the warmest

**Table 3.2** A comparison of life history traits in *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* after incubation at different temperatures during development.

| Effect | *P* | | | | |
|---|---|---|---|---|---|
| | Egg Hatchability | Larval Eclosion | Larval Development Time | Pupal Survival | Pupal Development Time |
| **Variation Among Strains** | | | | | |
| Experimental Temp* | <0.001 | 0.266 | <0.001 | <0.01 | <0.001 |
| (Experimental Temp)$^2$ | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Strain | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Strain*Experimental Temp | <0.001 | <0.001 | <0.001 | <0.001 | N/A |
| Strain*(Experimental Temp)$^2$ | <0.001 | <0.001 | <0.001 | <0.001 | N/A |
| **Adaptation to Temp** | | | | | |
| Experimental Temp | <0.001 | 0.266 | <0.001 | <0.01 | N/A |
| (Experimental Temp)$^2$ | <0.001 | <0.001 | <0.001 | <0.001 | N/A |
| Warm Native Temp | <0.001 | <0.001 | <0.05 | <0.001 | N/A |
| Cold Native Temp | <0.001 | <0.001 | <0.001 | <0.001 | N/A |
| Warm Native Temp* Experimental Temp | <0.001 | <0.001 | N/A | <0.001 | N/A |
| Cold Native Temp* Experimental Temp | <0.01 | <0.01 | N/A | <0.001 | N/A |
| Warm Native Temp* (Experimental Temp)$^2$ | <0.001 | N/A | N/A | <0.01 | N/A |
| Cold Native Temp* (Experimental Temp)$^2$ | <0.001 | N/A | N/A | N/A | N/A |

* Temp = Temperature

N/A = Term was not included in the minimally-adequate model.

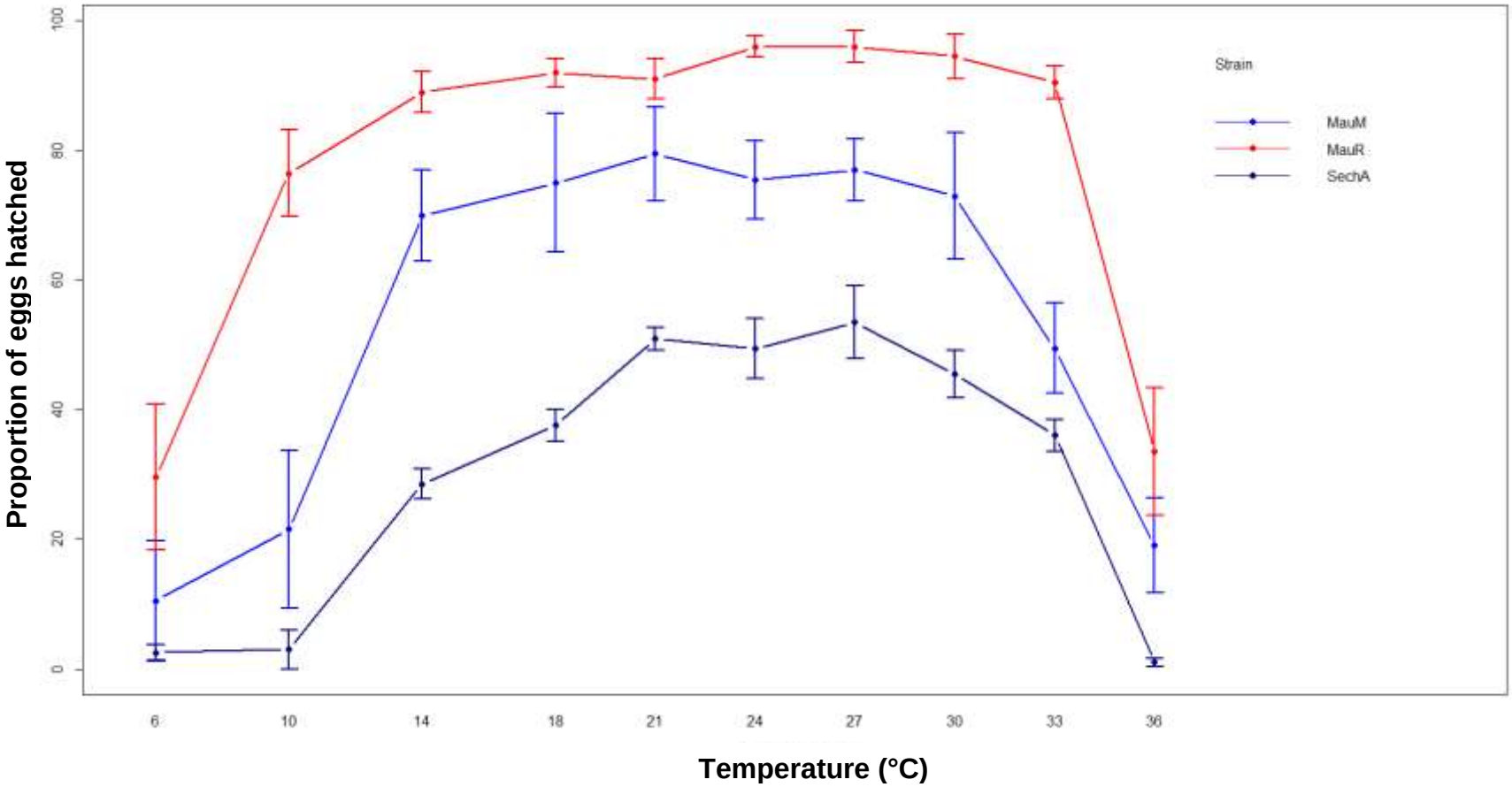


**Figure 3.1** Proportion of eggs hatched for *Drosophila mauritiana* and *D. sechellia* at temperatures from 6 – 36 °C. Strain names are reported on the right of each figure and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

(β = -13.840, df = 1, $\chi^2$ = 444.39, $P$ < 0.001) and coldest monthly native temperatures (β = 3.806, df = 1, $\chi^2$ = 134.36, $P$ < 0.001). Egg hatchability also depended on the interaction between the linear term for the experimental temperatures and the warmest monthly temperatures (β = 1.052, df = 1, $\chi^2$ = 28.13, $P$ < 0.001), the interaction between the linear term for the experimental temperature and the coldest monthly temperatures (β = -0.294, df = 1, $\chi^2$ = 10.83, $P$ < 0.01), the interaction between the quadratic term for the experimental temperature and the warmest monthly temperatures (β = -0.022, df = 1, $\chi^2$ = 76.47, $P$ < 0.001), and the interaction between the quadratic term for the experimental temperature and the coldest monthly temperatures (β = 0.006, df = 1, $\chi^2$ = 51.79, $P$ < 0.001).

After incubation at the egg stage, the adult dry mass depended on the experimental temperature (Table 3.3; GLZ; β = -2.117, df = 1, $\chi^2$ = 29910, $P$ < 0.05), the strain of the fly (df = 2, $\chi^2$ = 136594, $P$ < 0.001), the sex of the fly (β = -135.935, df = 1, $\chi^2$ = 607517, $P$ < 0.001), and the interaction between the strain and sex of the fly (df = 2, $\chi^2$ = 45712, $P$ < 0.05).

### 3.3.2 Larval response to temperature

I measured larval survival by incubating of a total of 1 600 adults for egg laying and removing 2 245 offspring from all 160 vials after eclosion (Figure 3.2; Appendix 13). Larval eclosion depended on the quadratic main effect of the experimental temperature (Table 3.2; GLZ; df = 1, $\chi^2$ = 989.00, $P$ < 0.001) and the strain of the fly (df = 3, $\chi^2$ = 2189.03, $P$ < 0.001). Larval survival also depended on the interaction between strain and the linear effect of experimental temperature (df = 3, $\chi^2$ = 89.52, $P$ < 0.001) and the interaction between strain and the quadratic effect of experimental temperature (df = 3, $\chi^2$ = 122.20, $P$ < 0.001). Larval eclosion did not depend on the main effect of the linear term for the experimental temperature (df = 1, $\chi^2$ = 1.24, $P$ = 0.266). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, larval eclosion depended on the quadratic main effect of the experimental temperature (β = -

**Table 3.3** A comparison of the effect of temperature incubation on the mass of *Drosophila mauritiana*, *D. sechellia*, and *D. nepalensis* during different life stages.

| **Effect** | ***P*** | | | |
|---|---|---|---|---|
| | Egg | Larvae | Pupae | Adult |
| **Variation Among Strains** | | | | |
| Experimental Temp* | <0.05 | <0.001 | <0.001 | <0.001 |
| Strain | <0.001 | <0.001 | <0.001 | <0.001 |
| Sex | <0.001 | <0.001 | <0.001 | <0.001 |
| Strain*Experimental Temp | N/A | N/A | N/A | N/A |
| Sex*Experimental Temp | N/A | N/A | N/A | <0.05 |
| Strain*Sex | <0.05 | <0.01 | <0.05 | <0.001 |

* Temp = Temperature

N/A = Term was not included in the minimally-adequate model

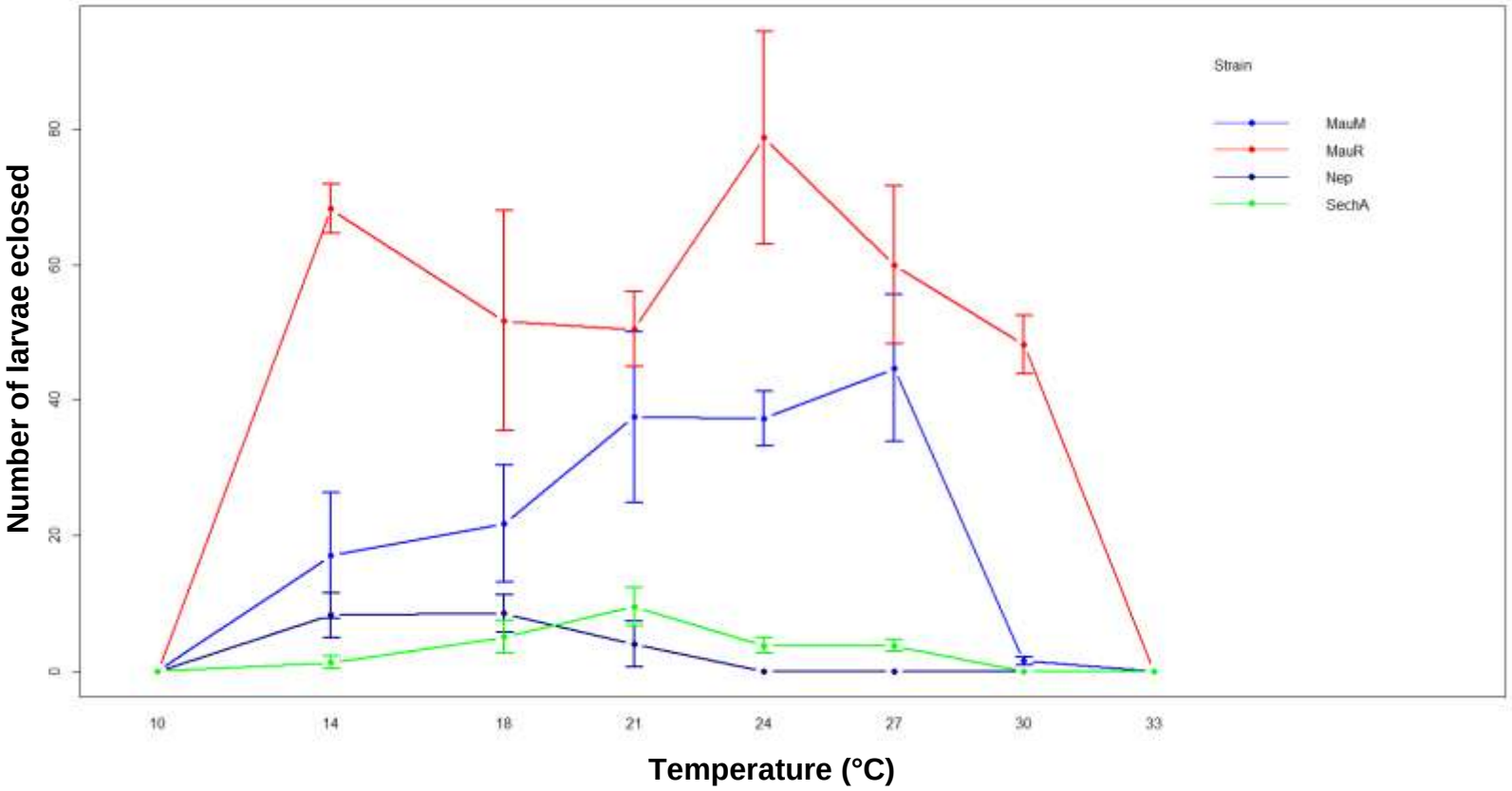


**Figure 3.2** Number of larvae eclosed for *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* at temperatures from 6 – 36 °C. Strain names are reported on the right of each figure and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

0.017, df = 1, $\chi^2$ = 989, $P$ < 0.001), and the warmest (β = -0.559, df = 1, $\chi^2$ = 383.16, $P$ < 0.001) and coldest monthly native temperatures (β = 0.352, df = 1, $\chi^2$ = 600.77, $P$ < 0.001). Larval survival also depended on the interaction between the linear term for the experimental temperature and the warmest monthly temperatures (β = 0.053, df = 1, $\chi^2$ = 47.25, $P$ < 0.001) and the interaction between the linear term for the experimental temperature and the coldest monthly temperatures (β = -0.019, df = 1, $\chi^2$ = 8.40, $P$ < 0.01). Larval eclosion did not depend on the main effect of the linear term for the experimental temperature (β = -0.222, df = 1, $\chi^2$ = 1.24, $P$ = 0.266).

Larval development time (Figure 3.3; Appendix 14) depended on the main effects of the linear (Table 3.2; GLZ; df = 1, $\chi^2$ = 4592.6, $P$ < 0.001) and quadratic effects of the experimental temperature (df = 1, $\chi^2$ = 980.9, $P$ < 0.001), and the strain of the fly (df = 3, $\chi^2$ = 107.7, $P$ < 0.001). The larval development time also depended on the interaction between strain and the linear effect of the experimental temperature (df = 3, $\chi^2$ = 80.8, $P$ < 0.001) and the interaction between strain and the quadratic effect of experimental temperature (df = 3, $\chi^2$ = 83.0, $P$ < 0.001). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, larval development time depended on the linear (Table 3.2; GLZ; β = -5.998, df = 1, $\chi^2$ = 4543.3, $P$ < 0.001) and quadratic effects of experimental temperature (β = 0.110, df = 1, $\chi^2$ = 944.5, $P$ < 0.001), and the warmest (β = 0.434, df = 1, $\chi^2$ = 23.1, $P$ < 0.05) and coldest monthly native temperatures (β = -0.016, df = 1, $\chi^2$ = 131.1, $P$ < 0.001).

After incubation at the larval stage, the adult dry mass depended on the linear effect of experimental temperature (GLZ; Table 3.3; β = -3.071, df = 1, $\chi^2$ = 38947, $P$ < 0.001), the strain (df = 3, $\chi^2$ = 87746, $P$ < 0.001), and the sex of the fly (df = 3, $\chi^2$ = 55412, $P$ < 0.001). The mass also depended on the interaction between the strain of the fly and experimental temperature (df = 3, $\chi^2$ = 14917, $P$ < 0.01).

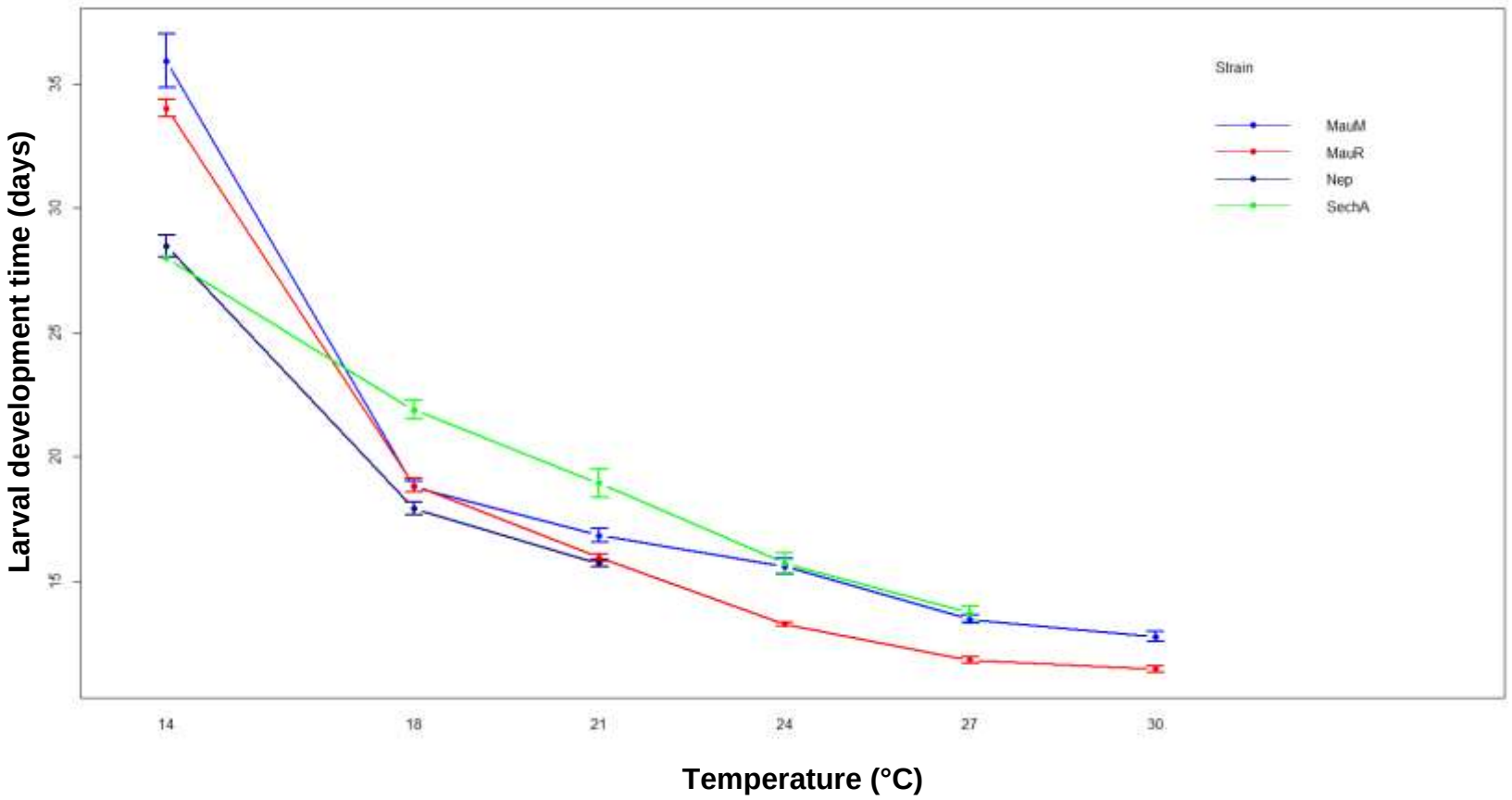


**Figure 3.3** Larval development time for *Drosophila mauritiana*, *D. sechellia*, and *D. nepalensis* at temperatures from 14 – 30 °C. Strain names are reported on the right of each figure and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature. *D. nepalensis* was not able to be tested at 24 - 30 °C because no flies survived until eclosion at these temperatures. *D. sechellia* was not able to be tested at 30 °C because no flies survived until eclosion at this temperature.

### 3.3.3 Pupal response to temperature

I measured pupal survival by incubating of a total of 3 200 third-instar larvae in 320 vials (Figure 3.4; Appendix 15). Pupal survival depended on the main effects of the linear (Table 3.2; GLZ; df = 1, $\chi^2$ = 7.96, $P$ < 0.01) and quadratic effects of experimental temperature (df = 1, $\chi^2$ = 1761.41, $P$ < 0.001), and the strain of the fly (df = 3, $\chi^2$ = 1344.17, $P$ < 0.001). Pupal survival also depended on the interaction between strain and the linear effect of experimental temperature (df = 3, $\chi^2$ = 220.10, $P$ < 0.001) and the interaction between strain and the quadratic effect of experimental temperature (df = 3, $\chi^2$ = 90.45, $P$ < 0.001). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, pupal survival depended on the main effects of the linear (Table 3.2; GLZ; β = 0.507, df = 1, $\chi^2$ = 7.96, $P$ < 0.01) and quadratic effects of experimental temperature (β = -0.089, df = 1, $\chi^2$ = 1824.83, $P$ < 0.001), and the warmest (β = -1.217, df = 1, $\chi^2$ = 436.72, $P$ < 0.001) and coldest monthly native temperatures (β = 0.868, df = 1, $\chi^2$ = 504.00, $P$ < 0.001). Pupal survival also depended on the interaction between the linear term for the experimental temperature and the warmest monthly temperatures (β = 0.086, df = 1, $\chi^2$ = 82.22, $P$ < 0.001), the interaction between the linear term for the experimental temperature and the coldest monthly temperatures (β = -0.075, df = 1, $\chi^2$ = 108.58, $P$ < 0.001), and the interaction between the quadratic term for the experimental temperature and the warmest monthly temperatures (β = 0.002, df = 1, $\chi^2$ = 7.79, $P$ < 0.01).

Pupal development time (Figure 3.5; Appendix 16) depended on the linear (Table 3.2; GLZ; β = -4.519, df = 1, $\chi^2$ = 1018.58, $P$ < 0.001) and quadratic effects of experimental temperature (β = 0.089, df = 1, $\chi^2$ = 349.86, $P$ < 0.001), and the strain of the fly (df = 3, $\chi^2$ = 98.05, $P$ < 0.001).

After incubation at the pupal stage, the adult dry mass depended on the linear effect of experimental temperature (GLZ; Table 3.3; β = -1.306, df = 1, $\chi^2$ = 16291, $P$ < 0.001), the strain (df = 3, $\chi^2$ = 53628, $P$ < 0.001), and the sex of the fly (β = -19.235, df = 1, $\chi^2$ =

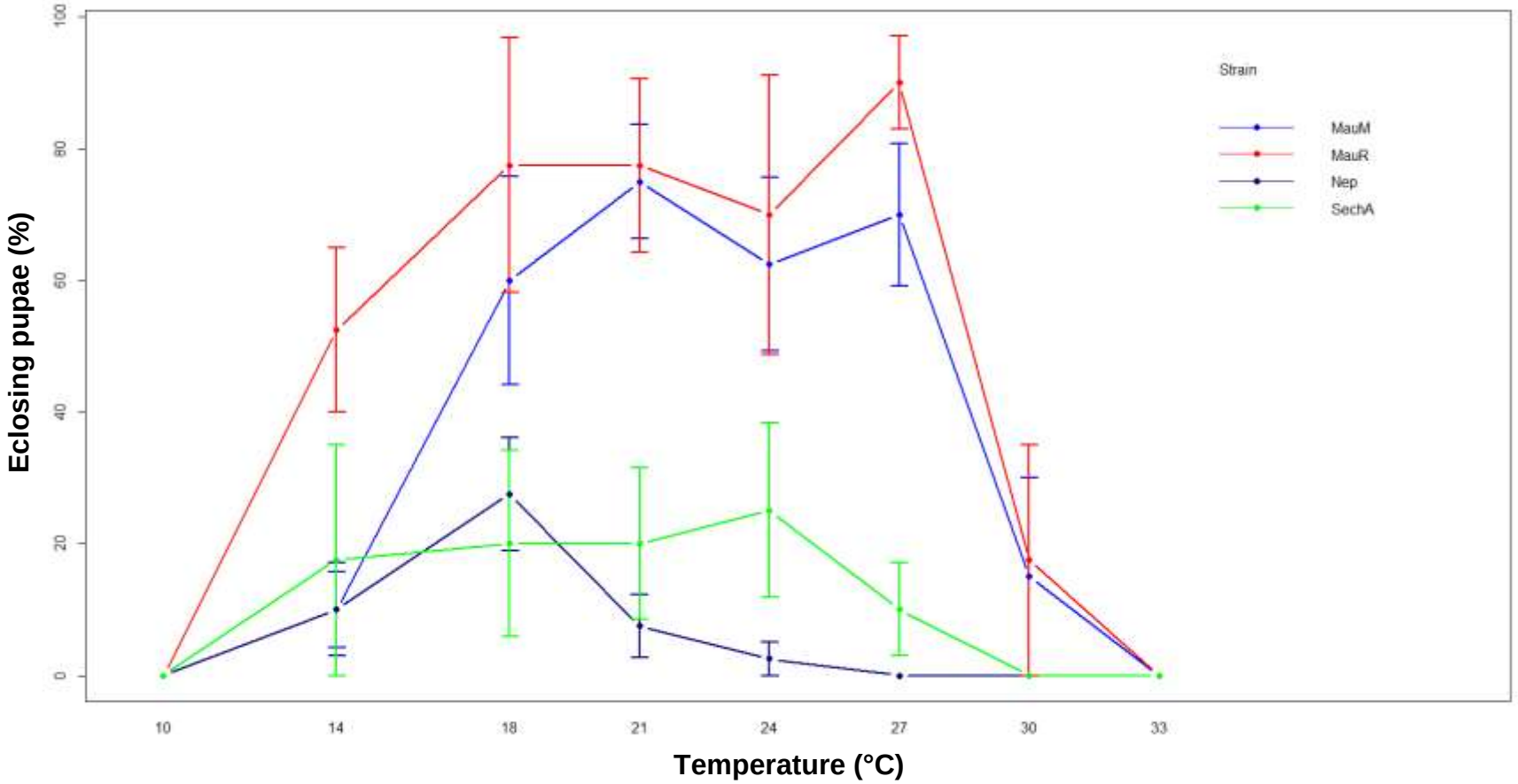


**Figure 3.4** Percent eclosion of pupae for *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* at temperatures from 6 – 36 °C. Strain names are reported on the right of each figure and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature.

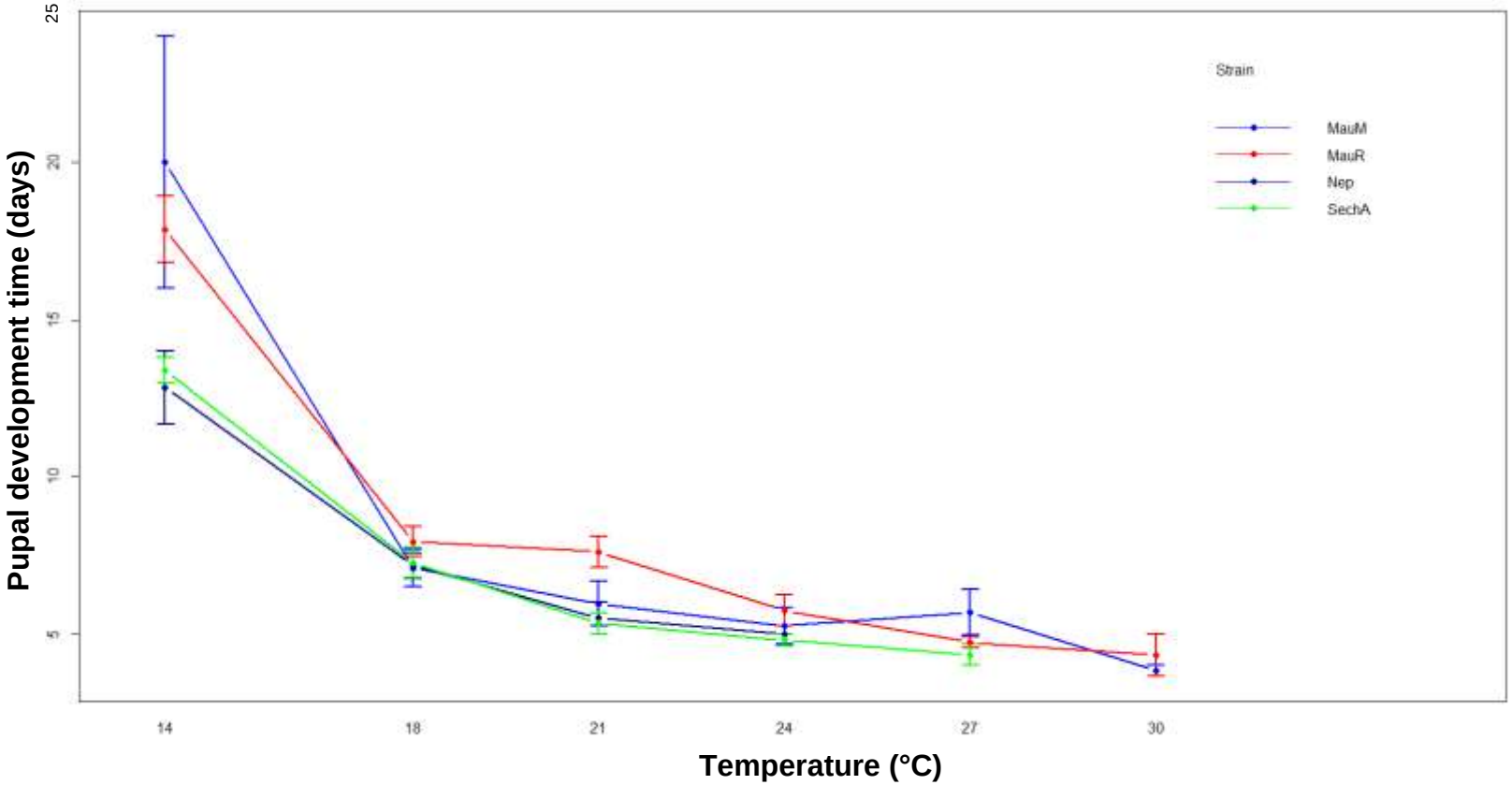


**Figure 3.5** Pupal development time for *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* at temperatures from 14 – 30 °C. Strain names are reported on the right of each figure and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature. *D. nepalensis* was not able to be tested at 27 - 30 °C because no flies survived until eclosion at these temperatures. *D. sechellia* was not able to be tested at 30 °C because no flies survived until eclosion at this temperature.

26878, $P < 0.001$). The mass also depended on the interaction between the strain and sex of the fly (df = 3, $\chi^2 = 10841$, $P < 0.05$).

### 3.3.4 Adult response to temperature for reproductive behaviours, mass, walking speed, and activity levels

I measured the incidence of courtship and copulation mating behaviours by observing a total of 800 pairs of flies (Figure 3.6; Appendix 17). Male courtship incidence depended on the main effects of the linear (Table 3.4; GLZ; df = 1, $\chi^2 = 8.47$, $P < 0.01$) and quadratic effects of experimental temperature (df = 1, $\chi^2 = 350.31$, $P < 0.001$), and the strain of the fly (df = 3, $\chi^2 = 49.57$, $P < 0.001$). Male courtship incidence also depended on the interaction between strain and the linear effect of experimental temperature (df = 3, $\chi^2 = 31.549$, $P < 0.001$) and the interaction between strain and the quadratic effect of experimental temperature (df = 3, $\chi^2 = 11.89$, $P < 0.01$). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, the male courtship incidence depended on the linear (Table 3.4; GLZ; $\beta = -0.641$, df = 1, $\chi^2 = 8.47$, $P < 0.01$) and quadratic effects of experimental temperatures ($\beta = -0.031$, df = 1, $\chi^2 = 326.47$, $P < 0.001$), and the warmest monthly native temperatures ($\beta = -2.212$, df = 1, $\chi^2 = 15.23$, $P < 0.001$). Male courtship incidence also depended on the interaction between the linear term for the experimental temperature and the warmest monthly temperatures ($\beta = 0.118$, df = 1, $\chi^2 = 20.43$, $P < 0.001$) and the interaction between the linear term for the experimental temperature and the coldest monthly temperatures ($\beta = -0.049$, df = 1, $\chi^2 = 4.65$, $P < 0.05$). Male courtship incidence did not depend on the main effect of the coldest monthly native temperatures ($\beta = 0.929$, df = 1, $\chi^2 = 1.32$, $P = 0.250$). The copulation incidence depended only on the linear effect of experimental temperatures (Figure 3.7; Appendix 18; Table 3.4; GLZ; df = 1, $\chi^2 = 23.032$, $P < 0.001$).

After incubation at the adult stage, the adult dry mass depended on the linear effect of experimental temperatures (GLZ; Table 3.3; $\beta = 3.270$, df = 1, $\chi^2 = 50229$, $P < 0.001$), the strain (df = 3, $\chi^2 = 330764$, $P < 0.001$), and the sex of the fly ($\beta = 6.824$, df = 1, $\chi^2 = 177913$, $P < 0.001$). The mass also depended on the interaction between the strain and

**Table 3.4** A comparison of life history traits in *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* after incubation at different temperatures during the adult life stage.

| Effect | *P* | | | |
|---|---|---|---|---|
| | Courtship | Copulation | Walking Speed | Activity Levels |
| **Variation Among Strains** | | | | |
| Experimental Temp* | <0.01 | <0.001 | <0.001 | N/A |
| (Experimental Temp)$^2$ | <0.001 | N/A | N/A | N/A |
| Strain | <0.001 | N/A | <0.001 | <0.001 |
| Sex | N/A | N/A | <0.01 | N/A |
| Strain*Experimental Temp | <0.001 | N/A | <0.001 | N/A |
| Strain*(Experimental Temp)$^2$ | <0.01 | N/A | N/A | N/A |
| Sex*Experimental Temp | N/A | N/A | <0.01 | N/A |
| Strain*Sex | | N/A | <0.001 | N/A |
| Strain*Experimental Temperature*Sex | | N/A | <0.001 | N/A |
| **Adaptation to Temp** | | | | |
| Experimental Temp | <0.01 | N/A | <0.001 | N/A |
| (Experimental Temp)$^2$ | <0.001 | N/A | | N/A |
| Warm Native Temp | <0.001 | N/A | <0.001 | N/A |
| Cold Native Temp | 0.250 | N/A | <0.01 | N/A |
| Sex | N/A | N/A | <0.05 | N/A |
| Warm Native Temp* Experimental Temp | <0.001 | N/A | N/A | N/A |
| Cold Native Temp* Experimental Temp | <0.05 | N/A | N/A | N/A |
| Experimental Temp*Sex | N/A | N/A | <0.05 | N/A |

* Temp = Temperature

N/A = Term was not included in the minimally-adequate model.

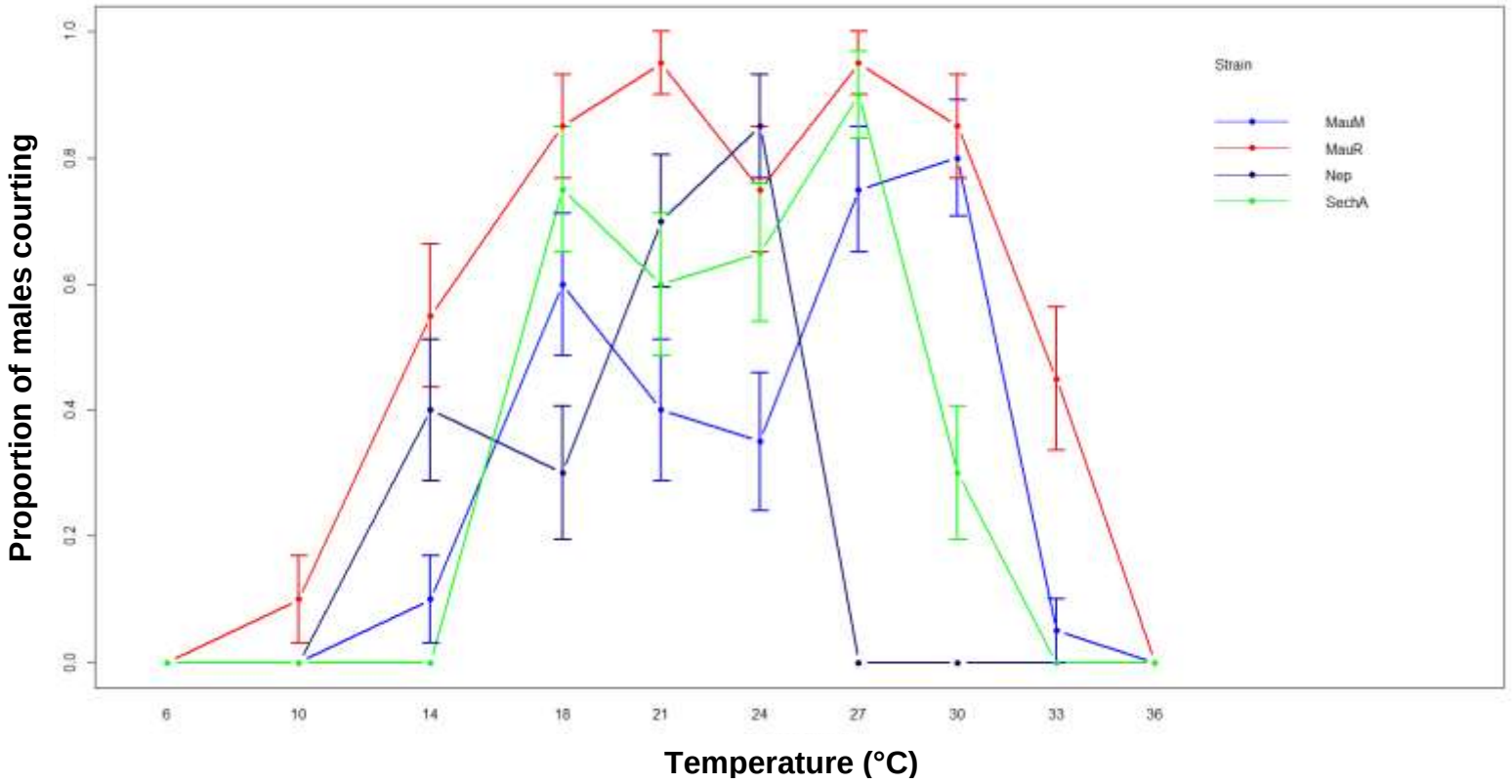


**Figure 3.6** Proportion of males courting during mating assays for *Drosophila mauritiana*, *D. sechellia*, and *D. nepalensis* at temperatures from 6 – 36 °C. Strain names are reported on the right of each figure and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature

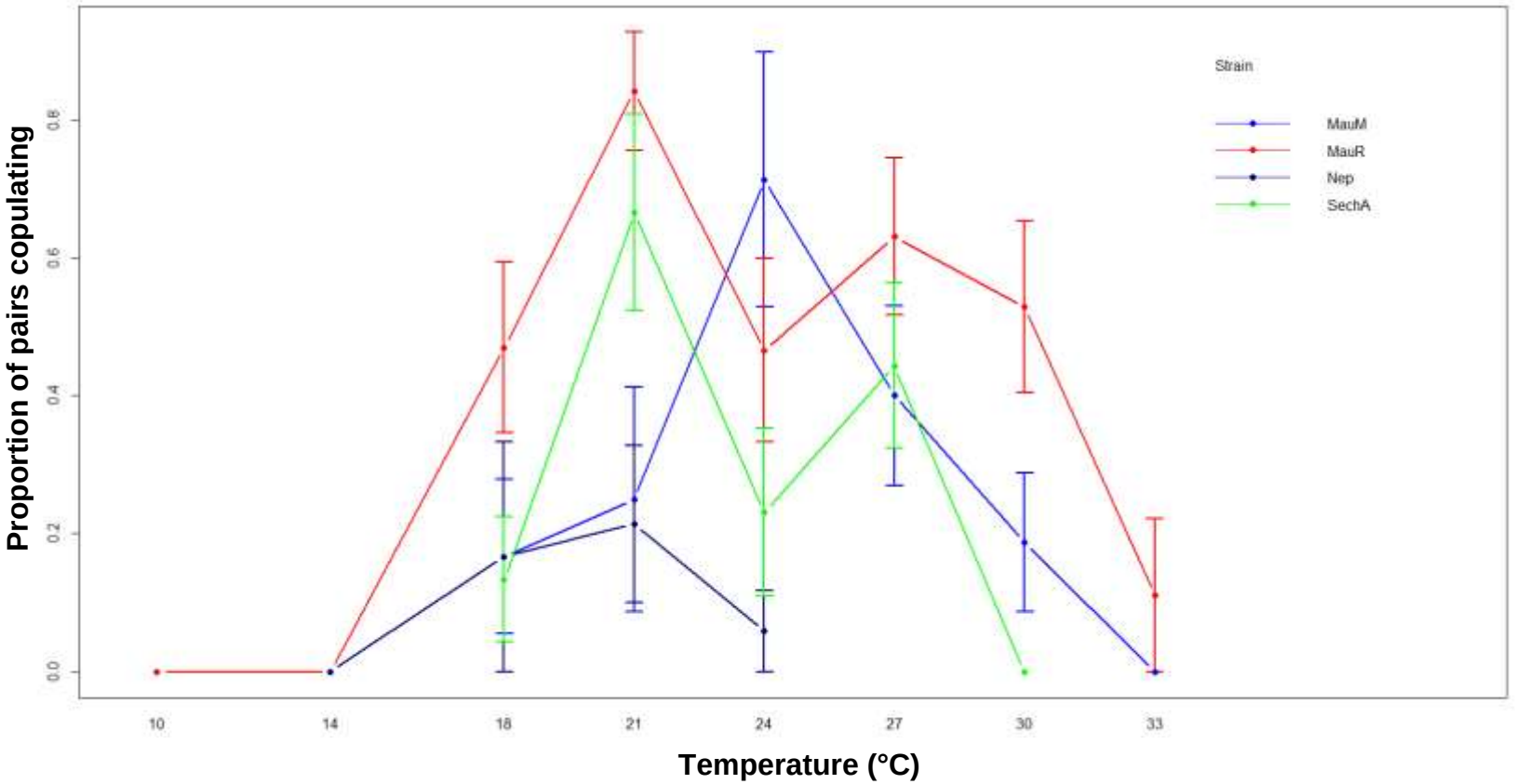


**Figure 3.7** Proportion of pairs copulating during mating assays for *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* at temperatures from 10 – 33 °C. Strain names are reported on the right of each figure and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature. *D. nepalensis* was not able to be tested at 27 - 33 °C because no flies survived at these temperatures. *D. sechellia* was not able to be tested at 10, 14, or 33 °C because no flies survived at this temperature.

sex of the fly (df = 3, $\chi^2$ = 35640, $P$ < 0.001) and the interaction between the sex of the fly and the linear effect of experimental temperatures (df = 1, $\chi^2$ = 9508, $P$ < 0.05). I measured the walking speed by observing a total of 288 individuals over the range of temperatures from 14 – 30 °C (Figure 3.8; Appendix 19). Walking speed depended on the linear main effect of experimental temperatures (GLZ; Table 3.4; df = 1, $\chi^2$ = 54.533, $P$ < 0.001), the strain of the fly (df = 3, $\chi^2$ = 73.741, $P$ < 0.001), and the sex of the fly (df = 1, $\chi^2$ = 6.468, $P$ < 0. 01). The walking speed also depended on the interaction between the strain and sex of the fly (df = 3, $\chi^2$ = 11.300, $P$ < 0.001), the interaction between the sex of the fly and the linear effect of experimental temperatures (df = 3, $\chi^2$ = 29.792, $P$ < 0.001), the interaction between the sex of the fly and the linear effect of experimental temperatures (df = 1, $\chi^2$ = 5.483, $P$ < 0.01), and the three-way interaction (df = 3, $\chi^2$ = 12.194, $P$ < 0.001). After reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, the walking speed depended on the linear effect of experimental temperatures (Table 3.4; GLZ; β = 0.057, df = 1, $\chi^2$ = 50.891, $P$ < 0.001), the sex of the fly (β = -0.884, df = 1, $\chi^2$ = 6.699, $P$ < 0.05), and the warmest (β = 0.523, df = 1, $\chi^2$ = 11.867, $P$ < 0.001) and coldest monthly native temperatures (β = -0.237, df = 1, $\chi^2$ = 7.516, $P$ < 0.01). The walking speed also depended on the interaction between the linear term for the experimental temperature and the sex of the fly (β = 0.055, df = 1, $\chi^2$ = 5.155, $P$ < 0.05).

I measured the activity level by observing a total of 288 individuals over the range of temperatures from 14 – 30 °C (Figure 3.9; Appendix 20). The walking speed depended only on the strain of the fly (Table 3.4; GLZ; df = 3, $\chi^2$ = 14.744, $P$ < 0.001).

### 3.3.5 Sex ratios

There was no effect of temperature on sex ratios at the egg (Table 3.5; GLZ; df = 1, $\chi^2$ = 0.011, $P$ = 0.637) or larval stages (GLZ; df = 1, $\chi^2$ = 0.015, $P$ = 0.520). After temperature incubation at the pupal stage, sex ratios were negatively affected by temperature (GLZ; df = 1, $\chi^2$ = 0.375, $P$ = 0.023). At low temperatures the sex ratio had a male bias, but the sex ratios were female biased at higher temperatures (Table 3.5).

**Table 3.5** Generalized linear model (GLZ) for comparison of sex ratios among experimental temperatures during the egg, larval and pupal life stages in *Drosophila mauritiana*, *D. sechellia*, and *D. nepalensis*.

| **Life Stage** | **Mean %♀** | **df** | $\chi^2$ | ***P*** |
|---|---|---|---|---|
| Egg | 48.0 | 1 | 0.011 | 0.637 |
| Larvae | 56.9 | 1 | 0.015 | 0.520 |
| Pupae | 51.7 | 1 | 0.375 | 0.023 |

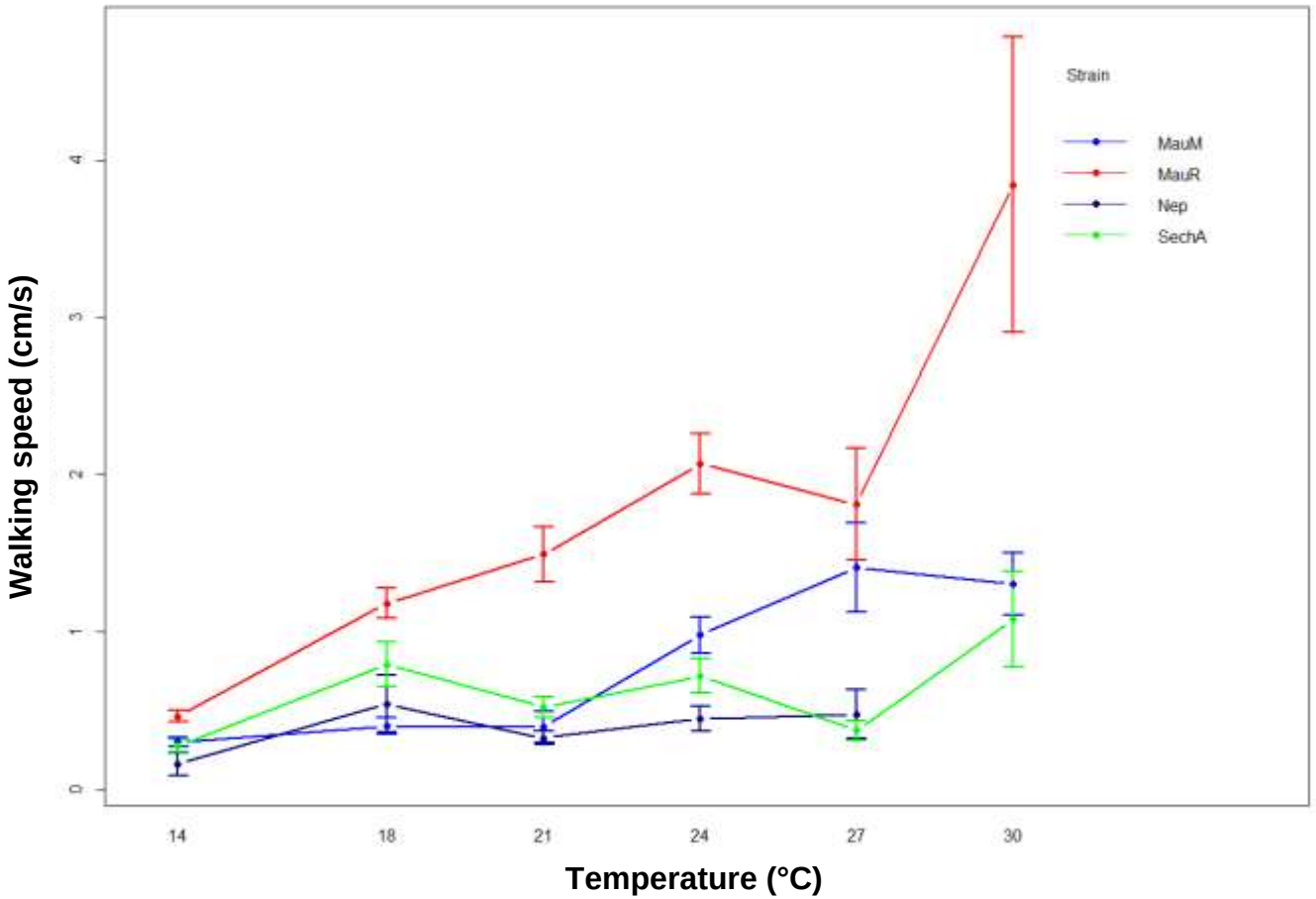


**Figure 3.8** The walking speed for *Drosophila mauritiana*, *D. sechellia*, and *D. nepalensis* at temperatures from 14 – 30 °C. Strain names are reported on the right of each figure and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature. *D. nepalensis* was not able to be tested at 30 °C because no flies survived at these temperatures.

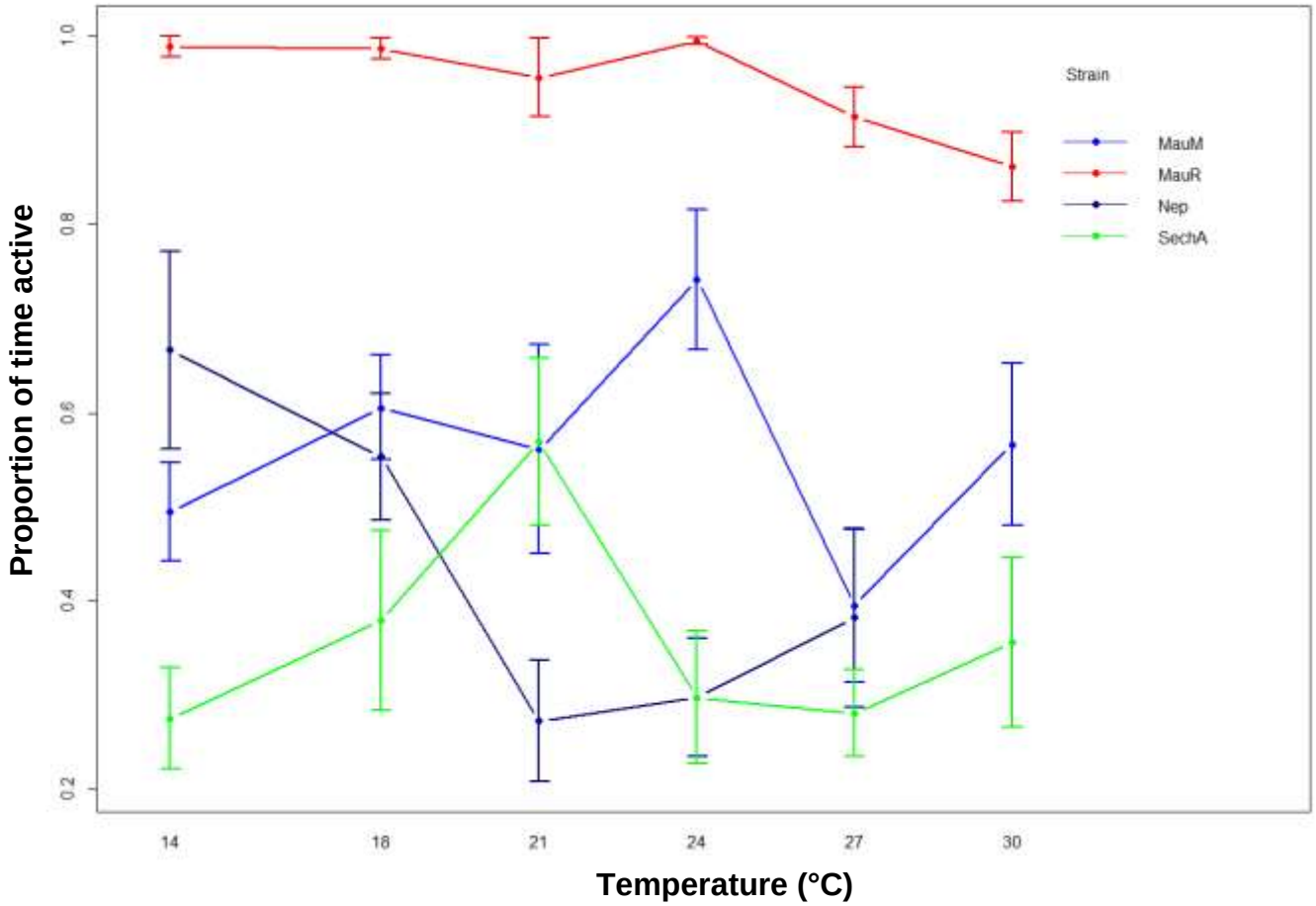


**Figure 3.9** The proportion of time activity during 30 s for *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* at temperatures from 14 – 30 °C. Strain names are reported on the right of each figure and represented by a line of that colour in the figure. Each point is the mean value (±SE) for that strain at that temperature. *D. nepalensis* was not able to be tested at 30 °C because no flies survived at these temperatures.

## 3.4 Discussion

This study investigated genetic differences in thermal tolerance in three species of *Drosophila* by comparing survival, reproduction, and performance at a variety of temperatures. My results show that across many life stages there are genetic differences in the way that *D. mauritiana*, *D. sechellia*, and *D. nepalensis* respond to a range of temperatures. In particular, in the life history traits related to survival at the egg, larval, and pupal stages, and for male courtship behaviour, there is variation among strains with respect to their response to experimental temperatures. For the other life history traits that did not show local adaptation, either phenotypic plasticity or a wide thermal tolerance may be responsible for this species persistence in each species' native environment. All three species that I tested are from geographically restricted locations such as islands and mountain ranges, where dispersal to new and more suitable habitats is possible, but more difficult compared to cosmopolitan species (David *et al.* 2007; Singh 2012; Parkash *et al.* 2013). My results agree with the results of past studies, which suggest that these three species are sensitive to changes in the environment and might be affected by climate change (Singh 2012). However, one must be cautious when drawing broad conclusions based on these data given the small number of isofemale lines I used for each species.

In general, the two *D. mauritiana* strains appeared to have a higher fitness than *D. sechellia* or *D. nepalensis* across most of the traits that I measured. My results are consistent with those of Strachan *et al.* (2012), which suggested that *D. mauritiana* was more tolerant to cold stress compared to *D. sechellia*, but are not consistent with the results of Nyamukondiwa *et al.* (2011), which suggest that the adults of *D. sechellia* are more tolerant than *D. mauritiana* to high- and low-temperature stress. Therefore, there are likely differences in thermal tolerance among life stages. Kellermann *et al.* (2012) studied the upper thermal limits of multiple *Drosophila* species when given a brief exposure to heat and found that *D. sechellia* had mid-level tolerance to heat relative to the rest of the genus, whereas *D. mauritiana* had very low tolerance to heat. That result contrasts with the results of this study, which found that *D. mauritiana* had higher tolerance to heat compared to *D. sechellia*. These seemingly conflicting results are

potentially explained if there is a different biological response to short-term (Kellermann *et al.* 2012) *vs*. long-term (my study) exposure to heat. *D. mauritiana* and *D. sechellia* seem to be sensitive to changes in temperature and might face extinction with continuing climate change and increasing temperatures.

My results also support previous studies of *D. nepalensis*, which have shown that this species is adapted to the low temperatures of the Himalaya Mountain range (Singh 2012; Parkash *et al.* 2013). Indeed, in my study *D. nepalensis* larvae could not develop at temperatures above 21 °C and could not survive at temperatures above 27 °C, which is a lower tolerance to warm temperatures than for any of the other species I tested. This result is consistent with those of previous studies comparing *D. nepalensis* to other close relatives (Parkash *et al.* 2013). Therefore, *D. nepalensis* appears to be sensitive to warm temperatures in the laboratory, which might reflect its response in its natural environment. These results are similar to past studies, which show that this species has a decreased fitness for multiple life history traits above 21 °C (Singh 2012; Parkash *et al.* 2013). However, the highest rate of courtship occurred at 24 °C, which is at the upper thermal limit for development. This surprising result might suggest that *D. nepalensis* mates and develops in different microclimates in its native environment. In contrast to courtship behaviour, copulation behaviour, which is primarily a measure of the receptiveness of females to their mating environment (Spieth 1974), only depended on inherent differences among strains and there was no significant effect of temperature (Figure 3.7).

If the responses to warm temperatures that I see in the laboratory reflect the response seen in natural populations, *D. nepalensis* will either have to evolve tolerance to warmer temperatures, continue to track cooler habitats higher in altitude, or perish as climate change continues. Although climate changes relatively slowly, *D. nepalensis* might be so intolerant to warmer environments that populations might not have sufficient time to evolve tolerance to warmer temperatures. Eventually the species will have retracted so far in altitude that the entire species might no longer have any suitable habitat and go extinct (Parkash *et al.* 2013). My results for *D. nepalensis* are similar to the results of Matute *et al.* (2009) conducted on another cold-adapted species, *D. santomea.* This species is

restricted to a mountain habitat on the island of São Tomé, where larvae can only develop at temperatures less than 28 °C (Matute *et al.* 2009). Its close relative, *D. yakuba*, is a warm-adapted species and lives at the base of these same mountains. The distributions of these two species overlap in the foothills and form a hybrid zone (Matute *et al.* 2009). Both *D. nepalensis* and *D. santomea* appear to be at risk for extinction with climate change due to their restricted habitat and cold-adapted nature.

In addition to the effects of temperature on survival and reproduction, there were also differences in the mass of the flies and the sex ratios after incubation at different temperatures. Although sex, strain, and experimental temperature differences were detected for mass in the three species of *Drosophila*, the lack of a significant interaction among these terms indicates that there is not local specialization to produce inherently heavier flies in a colder climate for a given strain, for example. After treatment at the pupal stage of development, there was also a significant skew in the sex ratio, independent of which strain was tested (Table 3.5). Therefore, all species show a similar shift from a male-biased sex ratio at low temperatures to a female-biased sex ratio with high temperatures. Although sex ratios were not affected by experimental temperature, this skew in sex ratios might affect population dynamics and size in *Drosophila* (Bateman 1948).

Interactions were detected between the experimental and native temperatures at each life stage of these species of *Drosophila*. These interactions reflect a correlation between the temperatures at which these species have the highest level of fitness in the laboratory and the native temperatures of the environments in which these species were sampled, which might indicate local adaptation to the thermal environment (Kawecki and Ebert 2004). Since this study only tested three species of *Drosophila*, created from a limited number of isofemale lines, I cannot definitively say that this trend applies broadly to species with geographically small-sized ranges (Garland and Adolph 1994; Table 3.1). However, these results are not likely a consequence of laboratory maintenance. Two of these species were collected within one year (*D. sechellia*) and one month (*D. mauritiana*) of this experiment, greatly reducing the chance of inbreeding or evolution towards fitness optima for laboratory conditions in these two species. Although *D. nepalensis* was

collected nearly a decade before this study began, its reaction norms indicate that the species still has a strong lack of tolerance to warm temperatures (Table 3.1), including those used in laboratory stock maintenance (22 – 24 ºC), suggesting that laboratory adaptation has not occurred in *D. nepalensis*. Therefore, detecting adaptation to native temperatures is possible even in strains that have been maintained in the laboratory for several years.

*D. nepalensis* flies were better able to tolerate cooler temperatures, while *D. mauritiana* flies from Rodrigues were able to tolerate warmer temperatures. Overall, the genetic differentiation exhibited by these species might allow for survival under the present climatic conditions, but with climate warming, some might go extinct. However, sufficient genetic variation for tolerance to warmer temperatures might be present in natural populations, and the presence of this variation could potentially be detected in a broader study than the one presented here. Species with geographically small sized ranges might face problems with tracking suitable habitats with climate change. Examining species with limited ranges for their response to experimental temperatures can allow us to determine the thermal limits of each species. Climate change models could them be used to predict how each species might respond to warming temperatures in their natural environment.

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# Chapter 4

# 4 General Discussion

## 4.1 Thermal biology of species of the *Drosophila melanogaster* species group

In Chapter 2, I reported that *D. melanogaster* showed local adaptation to temperature by detecting significant variation related to local climate at the larval and adult life stages. In Chapter 3, significant variation was identified among three species of the *D. melanogaster* species group with small geographic ranges in their response to experimental temperatures. Changes in experimental temperature affected the fitness of all of the species that I tested in the *D. melanogaster* species group at multiple life stages; this might reflect how these species respond to temperature in their native environment. Many species and populations that I examined have reduced fitness at warmer temperatures, which suggests that they might be at risk for extirpation of populations, and potentially extinction, if temperatures increase in their environment. However, additional tests of fitness are required to predict how these species will respond to climate change.

Across all life history traits that I tested there was a critical temperature at which performance declined to low levels, indicating that all species are sensitive to 30 °C changes in temperatures. For some populations, this range of temperatures is experienced over each annual cycle, which suggests that acclimatization might be important to the survival of flies. In addition, short-term exposures to the extreme ends of the temperature ranges used in this study might occur without affecting fitness to the same extent that long-term exposure does, perhaps through the use of phenotypic plasticity. Since populations and species are predicted to experience long-term exposure to extreme temperatures with climate change, there will be an overall decline in fitness. Laboratory selection experiments can be used to determine the ability of populations to evolve tolerance to long-term exposures of warmer temperatures, which might reflect the response that populations will have in their native environments.

Among all of the species I tested, two life stages showed consistent genetic variation among strains in response to temperature: the larval stage and the adult stage. I also detected variation for thermal tolerance among *D. melanogaster* populations during the adult stage when examining activity at different temperatures. In contrast, I detected variation for thermal tolerance among species with small-sized ranges during the egg and pupal stages. Therefore, different life stages are more sensitive to changes in temperature in the cosmopolitan species *D. melanogaster* (Chapter 2) and *D. simulans* (Austin and Moehring 2013) compared to species of *Drosophila* with geographically small-sized ranges (Chapter 3).

In the *D. melanogaster* species group, some interesting patterns emerged from studying how these species respond to a wide range of temperatures. Across all measures of survival, the Rodrigues Island strain of *D. mauritiana* seems to be the strain which has the greatest breadth of temperatures where fitness is not affected, which is surprising given the relatively warm native climate of the island and the narrow range of temperatures experienced by that population (Table 3.2). This strain was sampled at the same time, with roughly the same number of isofemale lines, as *D. sechellia* from the Seychelles and *D. mauritiana* from Île Maurice, neither of which shows the same breadth of thermal tolerance. Although *D. mauritiana* is an island species, the effective population size and the genetic diversity of *D. mauritiana* from Île Maurice are nearly as high as the mainland cosmopolitan *D. simulans* (Kliman *et al.* 2000). Since Rodrigues Island was colonized by *D. mauritiana* from Île Maurice, the *D. mauritiana* population on Rodrigues Island would be expected to have a lower, not greater, amount of genetic diversity than this founder population. The most likely explanation, therefore, is that there has been some gene flow from *D. simulans*, which tends to be more phenotypically plastic (Hodin and Riddiford 2000; David *et al.* 2004; Gibert *et al.* 2004) and that loci conveying some of this ability have introgressed into this population of *D. mauritiana*. Genome-wide sequence comparisons among laboratory and field strains of the two island populations of *D. mauritiana* and the Madagascar population of *D. simulans* would help determine whether introgression is occurring. Sequencing these three strains would also determine the extent to which my synthetic lines for each of these species tested reflect the actual genetic variation that is present in each of these populations.

Another comparison of interest is between the *D. melanogaster* and *D. sechellia* strains from the Seychelles. These strains reside in sympatry on the island, but potentially might experience different microclimatic conditions. *D. melanogaster* is a known human commensal and is usually found inside buildings (Lachaise and Silvain 2004). In contrast, *D. sechellia* is not a human commensal and is usually found on its host plant, *Morina citrifolia* (David *et al.* 2004; David *et al.* 2007). Thus, there might be potential for specialization to microclimates between the two species. In my study, both species appear to have the same levels of fitness along a range of experimental temperatures, indicating that adaptation to divergent microclimates has probably not occurred. Interestingly, both of these strains do have a relatively low overall survival or performance compared to the rest of the strains or species that I tested. This result might be indicative of inbreeding, either in their native environment or in the laboratory environment. While laboratory inbreeding is a distinct possibility for the *D. melanogaster* strain (collected in 1987), the strain of *D. sechellia* I used in my study was sampled the same year as experiments began (2012) and its poor performance across all tested temperatures is likely the result of inbreeding in its natural environment (Irvin *et al.* 1998). This is expected given that this species likely arose from a few colonizers from mainland Africa, which would result in a population bottleneck and lead to the low amount of genetic variation observed within this species (Irvin *et al.* 1998; Legrand *et al.* 2009). This same lack of variation is possible for the strain of *D. melanogaster* that was sampled from the Seychelles, which might have faced the same challenges upon arrival to the island; a genetic analysis of field populations of *D. melanogaster* is required to confirm this assertion.

A similar sympatric relationship exists between the strains of *D. melanogaster* and *D. mauritiana* from Île Maurice, Mauritius. For most traits, the two strains have approximately the same level of survival, with slightly wider tolerances to temperature by the *D. melanogaster* strain at the egg and adult stages. The wider thermal breadth for *D. melanogaster* might be a remnant of the ancestral cosmopolitan nature of these flies that have since become locally adapted for most traits to their local environment. One caveat to these comparisons, however, is that my study was based on very few isofemale lines, and thus I might not have captured all of the genetic variation in the population. Using a

greater number of lines might help accurately predict how these species would perform in the wild.

Comparisons can also be made between the two cosmopolitan species within this group, *D. melanogaster* and *D. simulans*. In *D. simulans*, only the larval stage had life history traits that were correlated with the local sampling environment (Austin and Moehring 2013; Appendix 11). In contrast, life history traits from both the larval and adult stages are correlated with local temperatures in *D. melanogaster* (Chapter 2). If these results are representative of the natural populations of *D. simulans*, then the species as a whole might not be as locally adapted as populations of *D. melanogaster* are to their native environments (Kawecki and Ebert 2004).

Lastly, when examining the incidence of copulation behaviour, the strains with geographically small ranges generally have narrower thermal breadths for performing mating behaviours compared to *D. melanogaster* (Chapter 2) and *D. simulans* (Austin and Moehring 2013). For example, the thermal breadth of courtship was approximately 9 °C for the species with geographically small sized ranges versus 16 °C for *D. melanogaster* and 13 °C for *D. simulans*. This narrower breadth means that reproduction might be thermally constrained in the strains with geographically small-sized ranges. Broad thermal tolerances are favoured when species are required to migrate or tolerate variable conditions, such as in temperate locations. Since mating behaviour is an important component of fitness, the mating behaviour of flies must remain fairly tolerant to a wide range of temperatures if individuals migrate or need to tolerate regions with variable or different climatic conditions (Angilletta 2009a).

## 4.2 Comparison of results to other studies

My thesis examined four species of the *D. melanogaster* species group across a wide range of temperatures and at multiple life stages. It is difficult to compare the results directly to the findings of other studies as the same range of temperatures and traits have not previously been examined together. Many previous studies examined a single aspect of thermal adaptation across a larger number of species (Addo-Bediako *et al.* 2000; Markow *et al.* 2009; Nyamukondiwa *et al.* 2011; Strachan *et al.* 2011; Kellermann *et al.*

2012). For example, Kellermann *et al.* (2012) examined a single trait, upper thermal limits, across 95 species of *Drosophila*. Of the four species from the *D. melanogaster* subgroup that were examined in my study, Kellermann *et al.* (2012) found that *D. melanogaster* was the most tolerant to heat stress, and *D. mauritiana* was the least tolerant, with *D. sechellia* and *D. simulans* displaying comparably intermediate heat tolerances (*D. nepalensis* was not included in their analysis). These results are in contrast to the results of my study which found that the survival of *D. mauritiana* was higher than the survival of *D. sechellia* at warmer temperatures, across multiple life stages. Other studies have examined multiple life history traits within a few strains or species of *Drosophila* (Overgaard *et al.* 2011; Parkash *et al.* 2013). Overgaard *et al.* (2011) measured the cold tolerance of multiple Australian species of *Drosophila*. The results of their study are similar to my own: the thermal tolerance of widespread species of *Drosophila* was greater than those with smaller range sizes (Overgaard *et al.* 2011).

The effect of extreme temperatures on fitness has been extensively studied (Guerra *et al.* 1997; Hoffmann *et al.* 2002; Noory *et al.* 2007; Kellermann *et al.* 2012), whereas fewer studies have focused on the fitness response of *Drosophila* to an intermediate range of temperatures (David *et al.* 2004; Austin and Moehring 2013). Studies that examine the effects of exposure to extreme temperatures generally conclude that extreme temperature tolerance determines the ability for species to persist in a particular environment. However, the fitness responses to changes in intermediate temperatures are biologically relevant because, with climate change, ectotherms will first be exposed to small changes in temperature and their ability to adapt to these initial shifts will determine their survival and fitness. Intermediate temperatures are often where performance is maximized for many life history traits, making it important to understand how severe the consequences of moving away from an optimum will be with climate change (Angilletta *et al.* 2002). Understanding the fitness consequences of shifts in intermediate temperatures might enable us make predictions about adaptive constraints in the presence of ongoing climate change. In this study, I found that the fitness of the *Drosophila* species that I tested was affected by the maintenance of these species at constant intermediate temperatures that they could experience in their native environment, based on local weather station data (Table 2.1; Table 3.1). Additional work examining the effect of fluctuating temperatures,

which is another method of temperature treatment that is even more biologically relevant, might provide additional support to my conclusions on the effects of temperature in the *D. melanogaster* group.

The effect of an intermediate range of temperatures on egg hatchability, larval survival, development time, fecundity, adult longevity, and body size have been examined individually in *Drosophila* (Murphy *et al.* 1983; Morin *et al.* 1996; James *et al.* 1997; Gibert *et al.* 2001; Gibert *et al.* 2004; Trotta *et al.* 2006; Matute *et al.* 2009). For example, Trotta *et al.* (2006) examined multiple traits, including body size, development time, pre-adult survival, longevity and reproductive success at temperatures from 12 – 31.2 °C in four populations of *D. melanogaster*. The researchers found that there were adaptive differences in tolerance, where populations from warm environments tolerated warmer temperatures better than populations from cooler temperatures. My results are consistent with these findings and confirm that local adaptation occurs in *D. melanogaster* across a wider geographic scale than was examined by previous studies. In addition, James *et al.* (1997) examined multiple populations of *D. melanogaster* for body size and development time across a range of intermediate temperatures. The researchers also found latitudinal clines for both traits. I did not use clinal variation to detect variation among populations, but my study did detect differences in body size among populations, which is consistent with the results of the study by James *et al.* (1997).

When researchers combined physiological measures of life history traits and range boundaries in multiple insect species from different orders, they found that many tropical species are living very close to their upper thermal boundaries and are experiencing declines in populations where climates are changing (Parmesan *et al.* 1999; Addo-Bediako *et al.* 2000; Deutsch *et al.* 2008; Sunday *et al.* 2012). Most of the species in my study that are living in tropical locations are from regions where summer temperatures exceed the temperatures at which their performance maximized, and are therefore potentially living near their upper thermal limit. These species might be finding microenvironments that are more suitable in their native environment, although their ability to find suitable habitats might be limited in very hot climates (Gibbs *et al.* 2003). Other widespread species of moths, spittlebugs, phyllids, and other insects are more

tolerant to changes in temperature than their close relatives that have restricted ranges (Bale *et al.* 2002; Butterfield and Coulson 1997). For example, diving beetle species that are widespread are more tolerant to high temperatures compared to related species that are restricted to mountain habitats (Calosi *et al.* 2008). My results agree with these past studies of other insect species, which suggests that, in general, widespread species are at a lower risk for extinction than species with smaller-sized ranges in other taxa than just *Drosophila* in the face of increasing temperatures due to climate change.

## 4.3 Future work

I have addressed thermal adaptation by testing many life history characters in *D. melanogaster* and examined the thermal biology of the three species of the *D. melanogaster* species group. The entire *D. simulans* complex has now been comprehensively tested for thermal tolerance (Chapter 3; Austin and Moehring 2013). However, many other species that are closely related to *D. melanogaster* and *D. simulans* remain to be tested for thermal adaptation. This includes the cosmopolitan species *D. ananassae*. This warm-adapted species is found across a large geographic range and is currently encroaching on the habitat previously occupied by *D. nepalensis* (Markow and O'Grady 2005; Parkash *et al.* 2013). It would be interesting to compare the thermal response of many populations sampled across the entire range of *D. ananassae* to see whether this species is thermally adapted to its environment across the entire distribution. These results could be compared with my results for *D. melanogaster* and *D. simulans*, which are also both cosmopolitan, to see if cosmopolitan species in general respond to temperatures in a similar manner. Additionally, examining other species of the *D. melanogaster* species subgroup with relatively small geographic ranges, including *D. teissieri*, *D. erecta*, and *D. orena*, would provide a comprehensive assessment of the way that *Drosophila* with restricted ranges respond to shifts in temperature.

In my study I investigated the thermal biology of multiple species of the *D. melanogaster* species group. However, this framework for studying the thermal tolerance can be used to study other insects and ectotherms. Insects are at a very high risk for extinction with climate change since their body temperatures closely follow that of the ambient environment. Given that there are over a million species of insects, and their critical role

in the food web, understanding how susceptible insects are to changing temperatures in their environment is important to preserve the biodiversity of our planet. Overall, many insects are shifting their range poleward (Hill *et al.* 2011). For instance, shifts have been observed in the ranges of temperate Odonata species (the order containing carnivorous insects) in the United Kingdom. Odonata have shifted 74 km Northward over a period of 40 years as a result of warming temperatures (Hickling *et al.* 2005). However, we know very little about the thermal biology of Odonata compared to *Drosophila* (Nilsson 2012). Two important components are required to accurately predict how species will respond to climate change: the physiological response to a range of temperatures and the capacity for species to adapt to changing temperatures (Chown *et al.* 2010). Many studies that track changes in distribution do not consider the ability of the population to adapt to changes in their environment (Angilletta 2009b; Chown *et al.* 2010). Therefore, a focus of future studies of insects should consider the adaptive ability of the organisms in their natural environment, particularly in their upper thermal tolerance (Neven *et al.* 2000). Species with both widespread and small-sized ranges seem to be sensitive to changes in native temperatures, and a failure of these species to adapt to increasing temperatures with climate change will likely result in loss of biodiversity.

## 4.4 References


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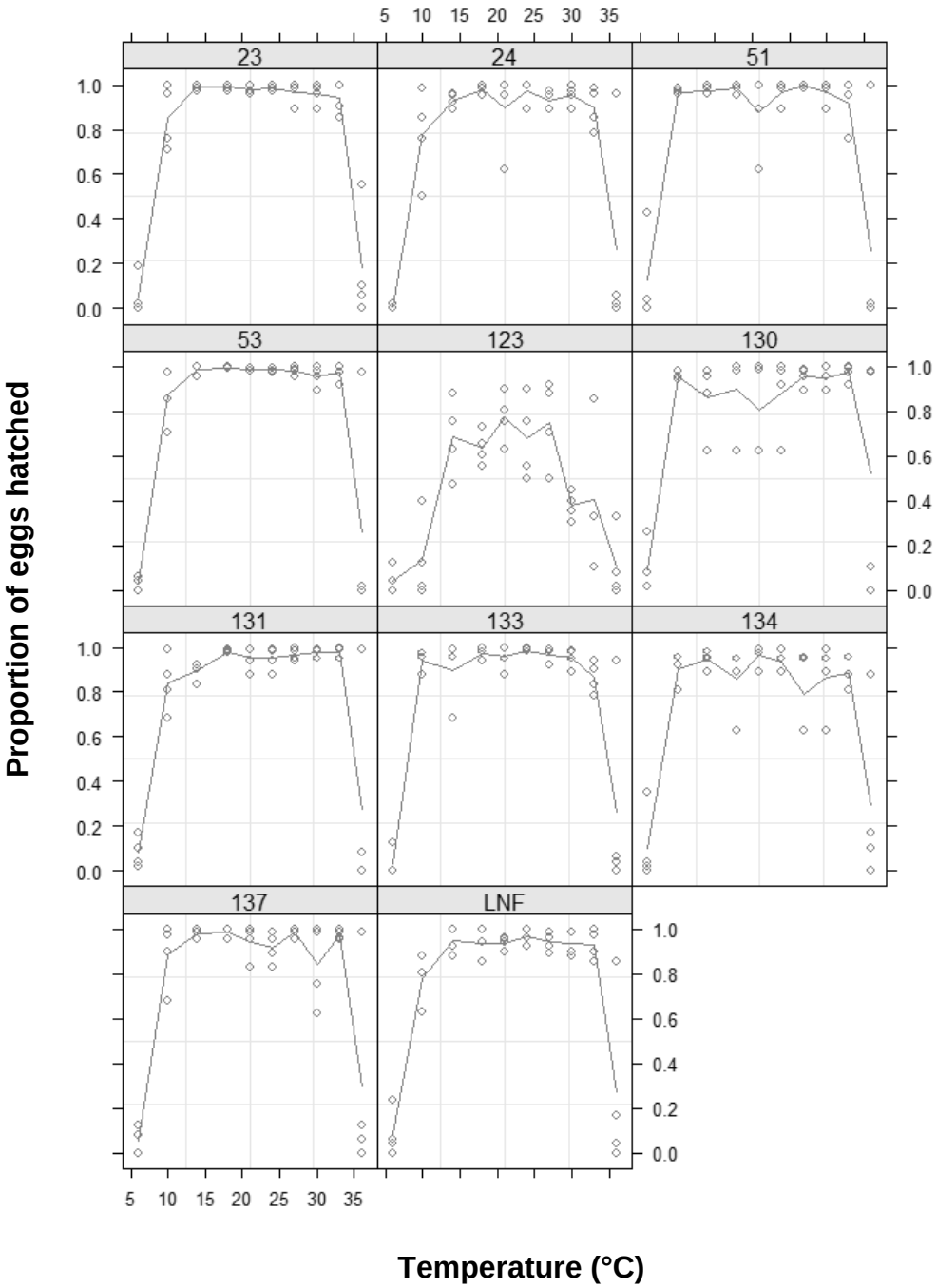
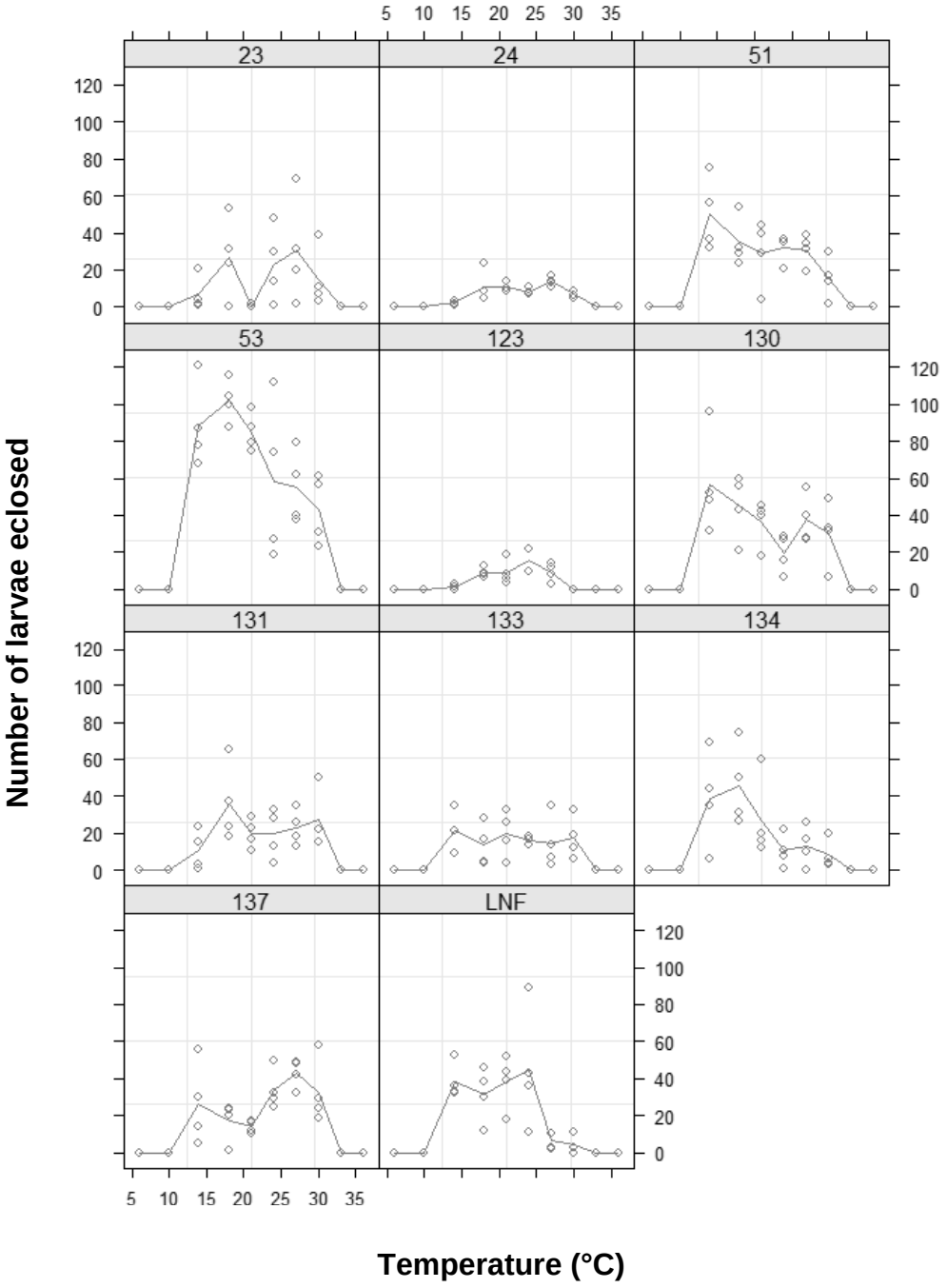
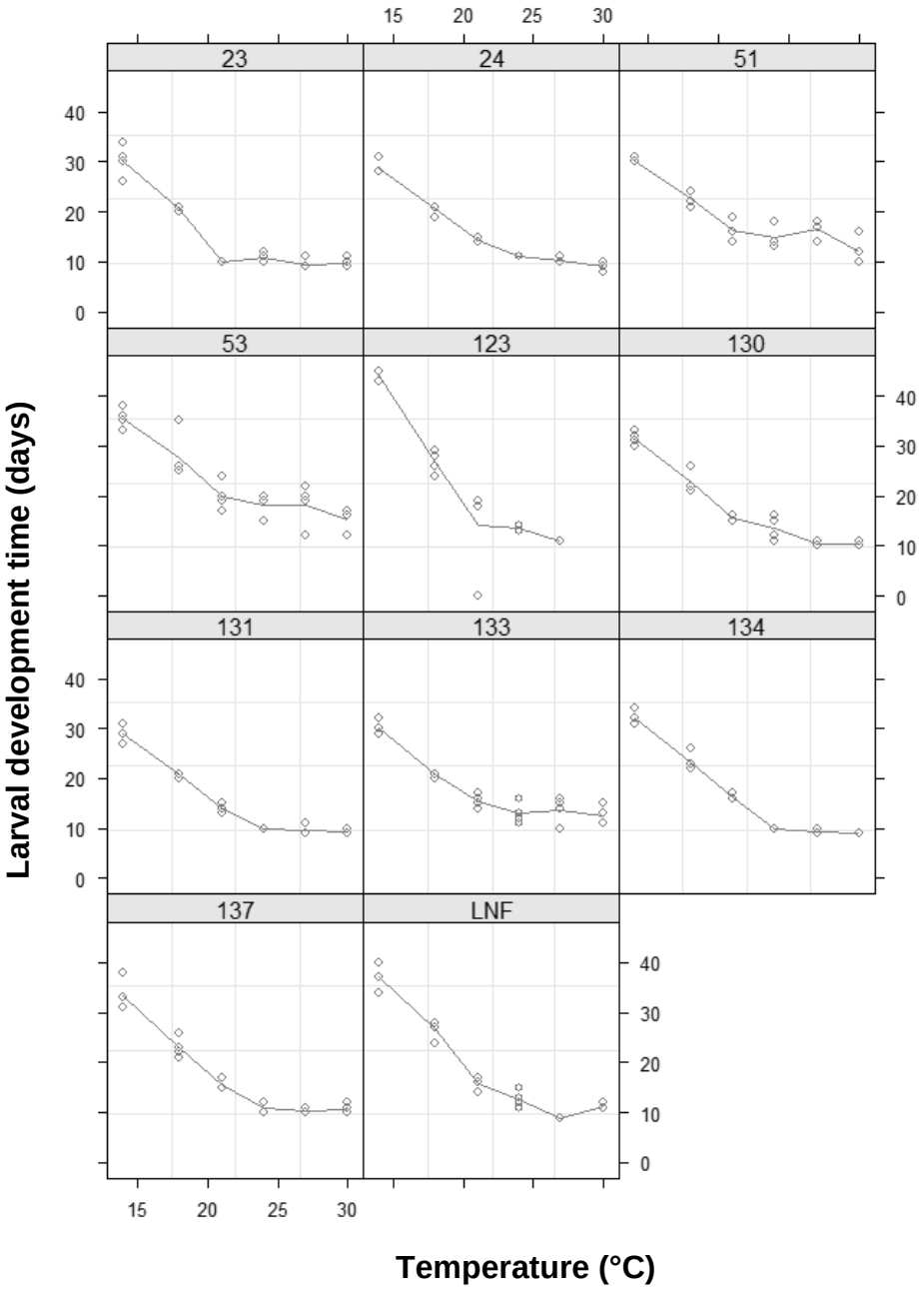
# Appendices



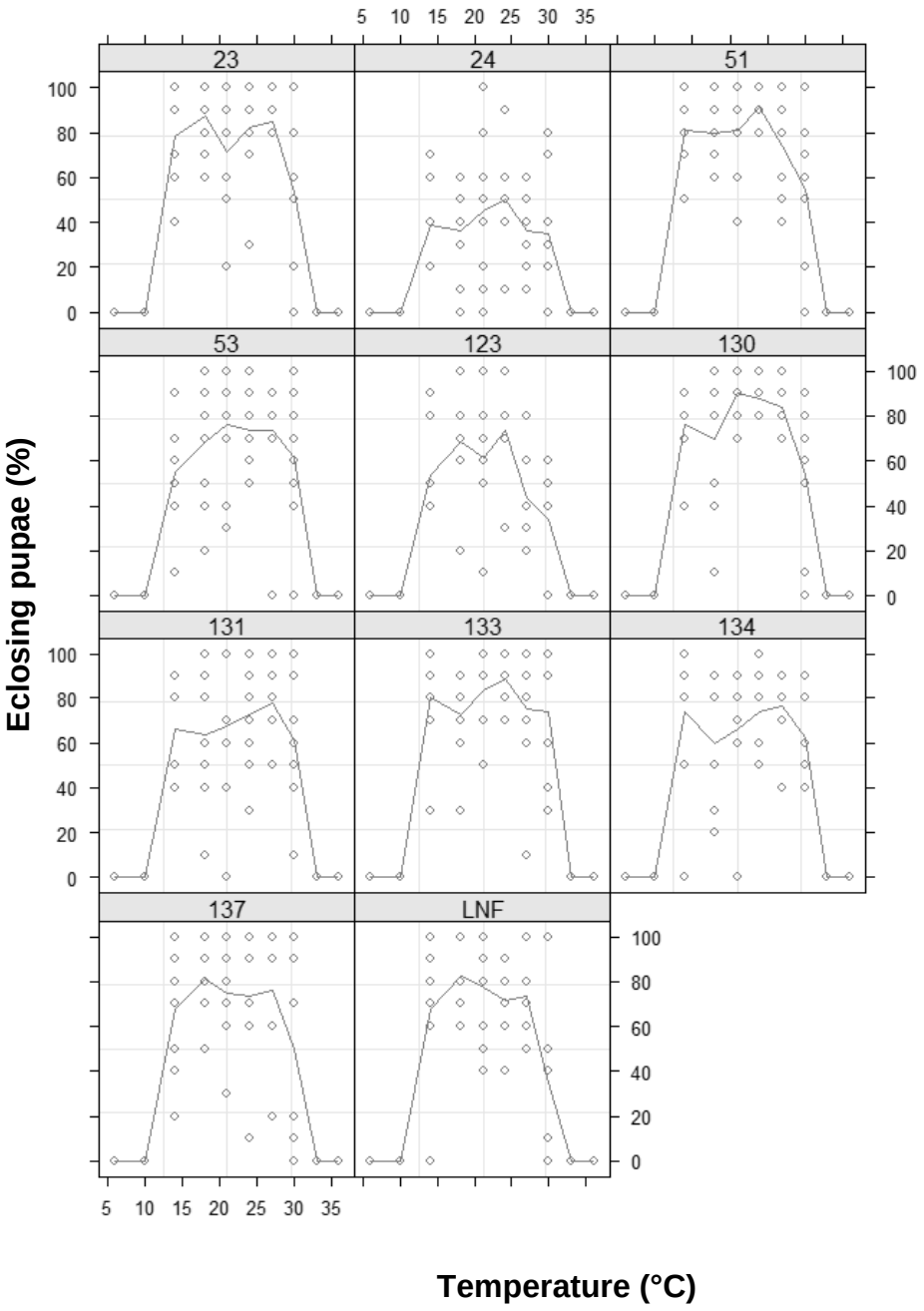

**Appendix 1.** Proportion of eggs hatched for eleven strains of *D. melanogaster* sampled from across the globe at temperatures from 6 – 36 °C. Diamonds represent the proportion of eggs that hatched out of fifty eggs. Strain numbers are reported at the top of each panel. The line is the mean response for each strain at each temperature.



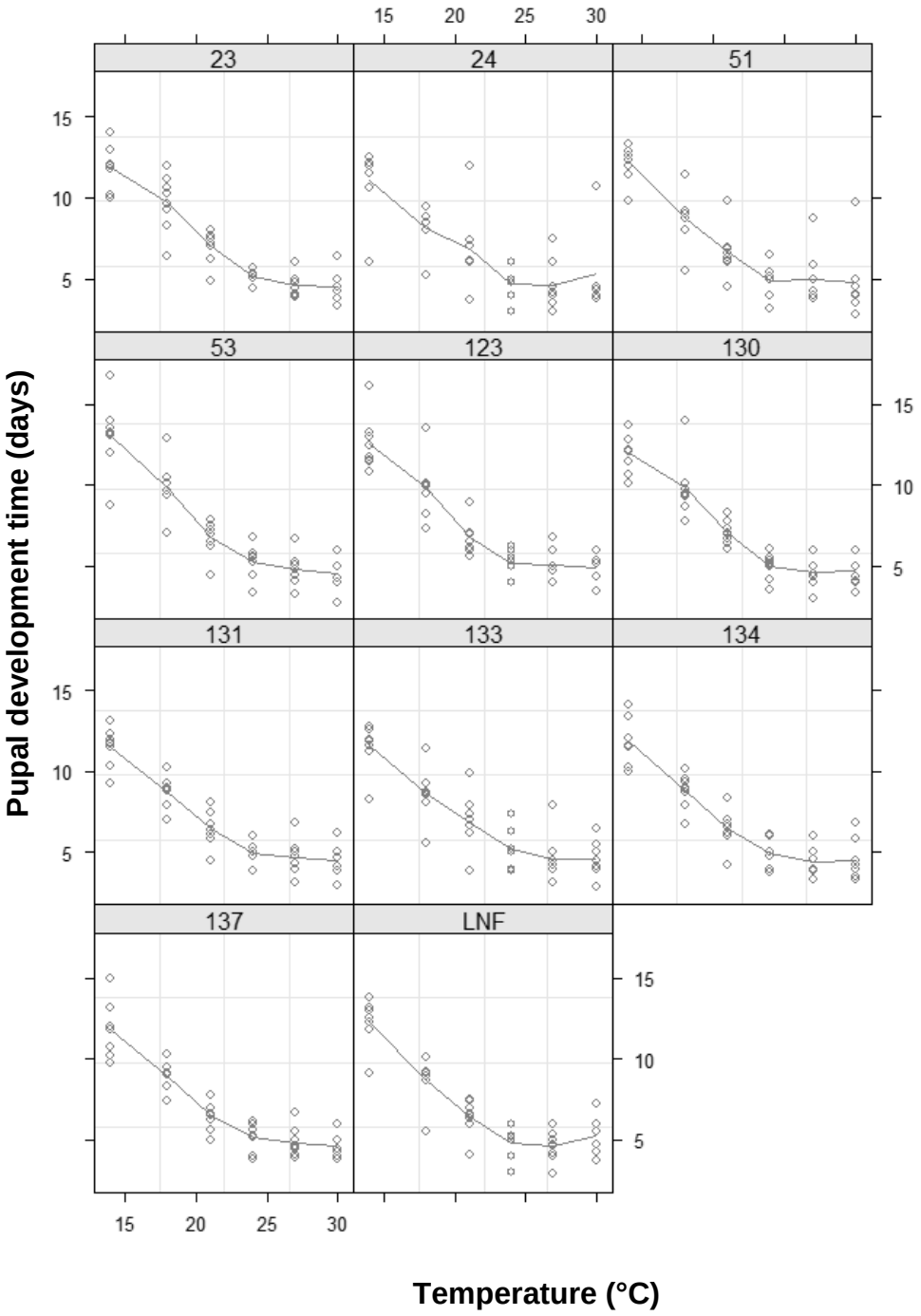

**Appendix 2.** Number of larvae eclosed for eleven strains of *D. melanogaster* sampled from across the globe at temperatures from 6 – 36 °C. Diamonds represent the number of larvae that eclosed from each vial. Strain numbers are reported at the top of each panel. The line is the mean response for each strain at each temperature.



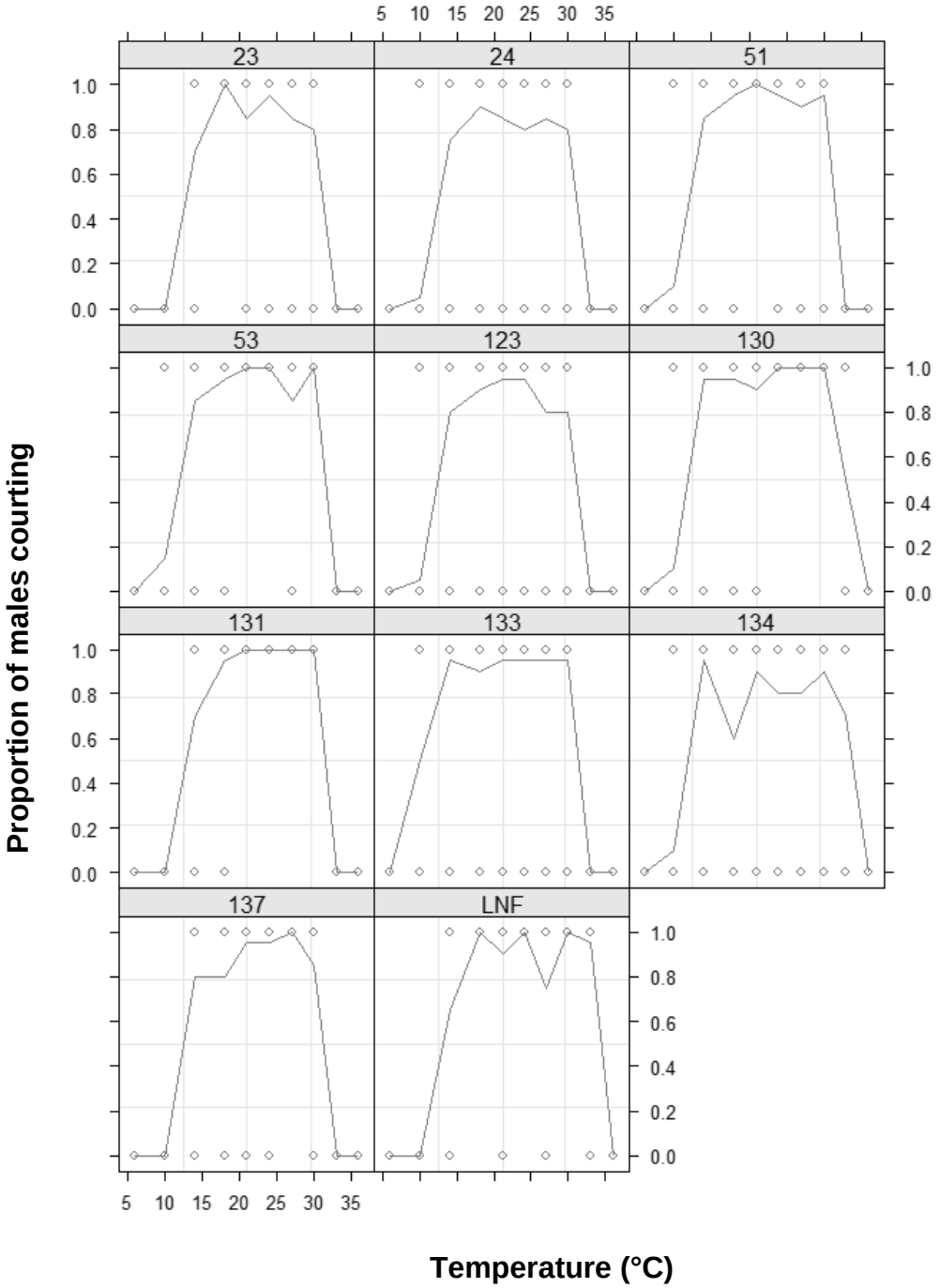

**Appendix 3.** Larval development time for eleven strains of *D. melanogaster* sampled from across the globe at temperatures from 14 – 30 °C. Diamonds represent the mean development time of the larvae that eclosed from each vial. Strain numbers are reported at the top of each panel. The line is the mean response for each strain at each temperature.



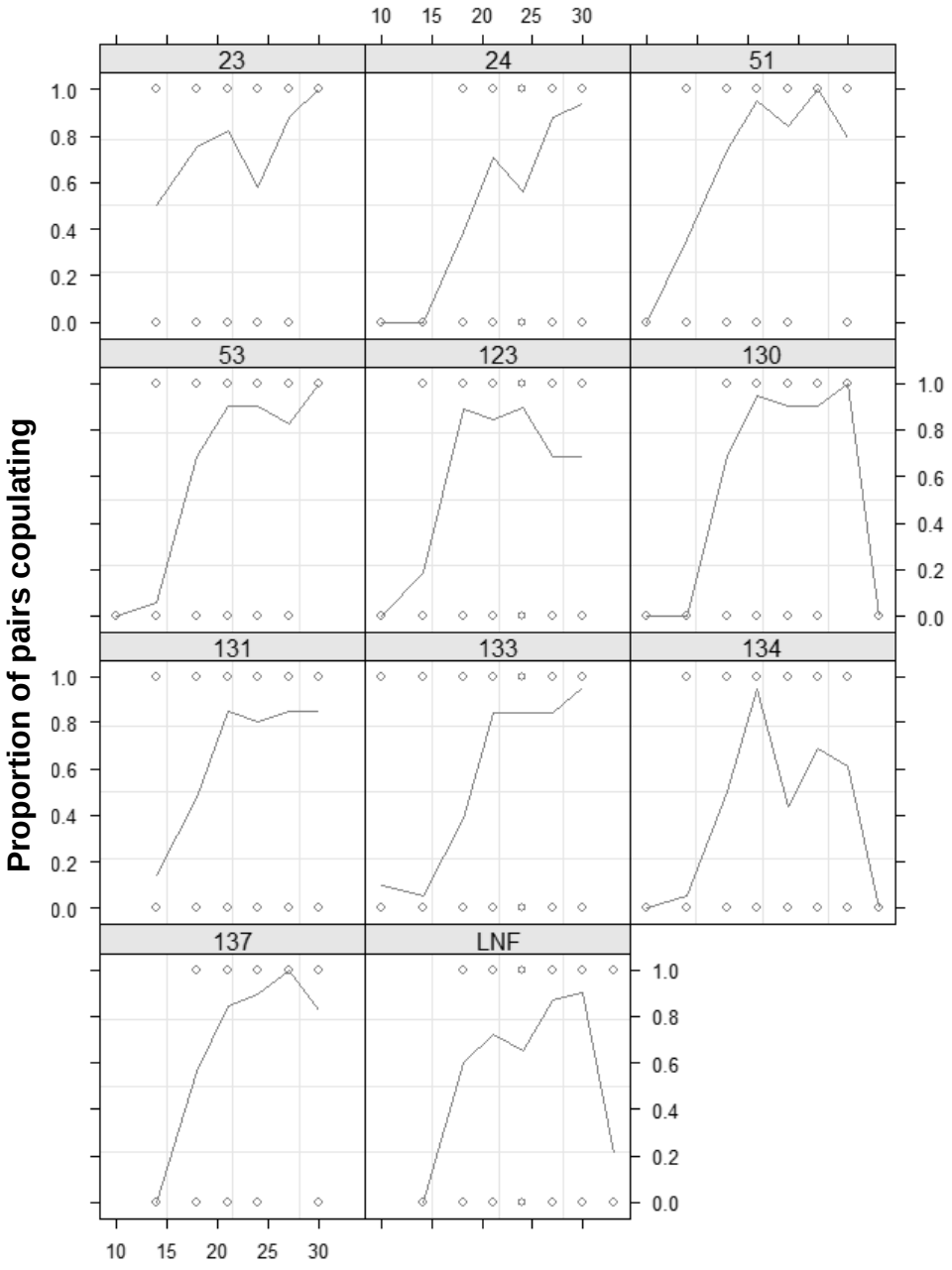

**Appendix 4.** Percent eclosion of pupae for eleven strains of *D. melanogaster* sampled from across the globe at temperatures from 6 – 36 °C. Diamonds represent the portion of pupae that eclose from each vial out of ten initial third-instar larvae. Strain numbers are reported at the top of each panel. The line is the mean response for each strain at each temperature.



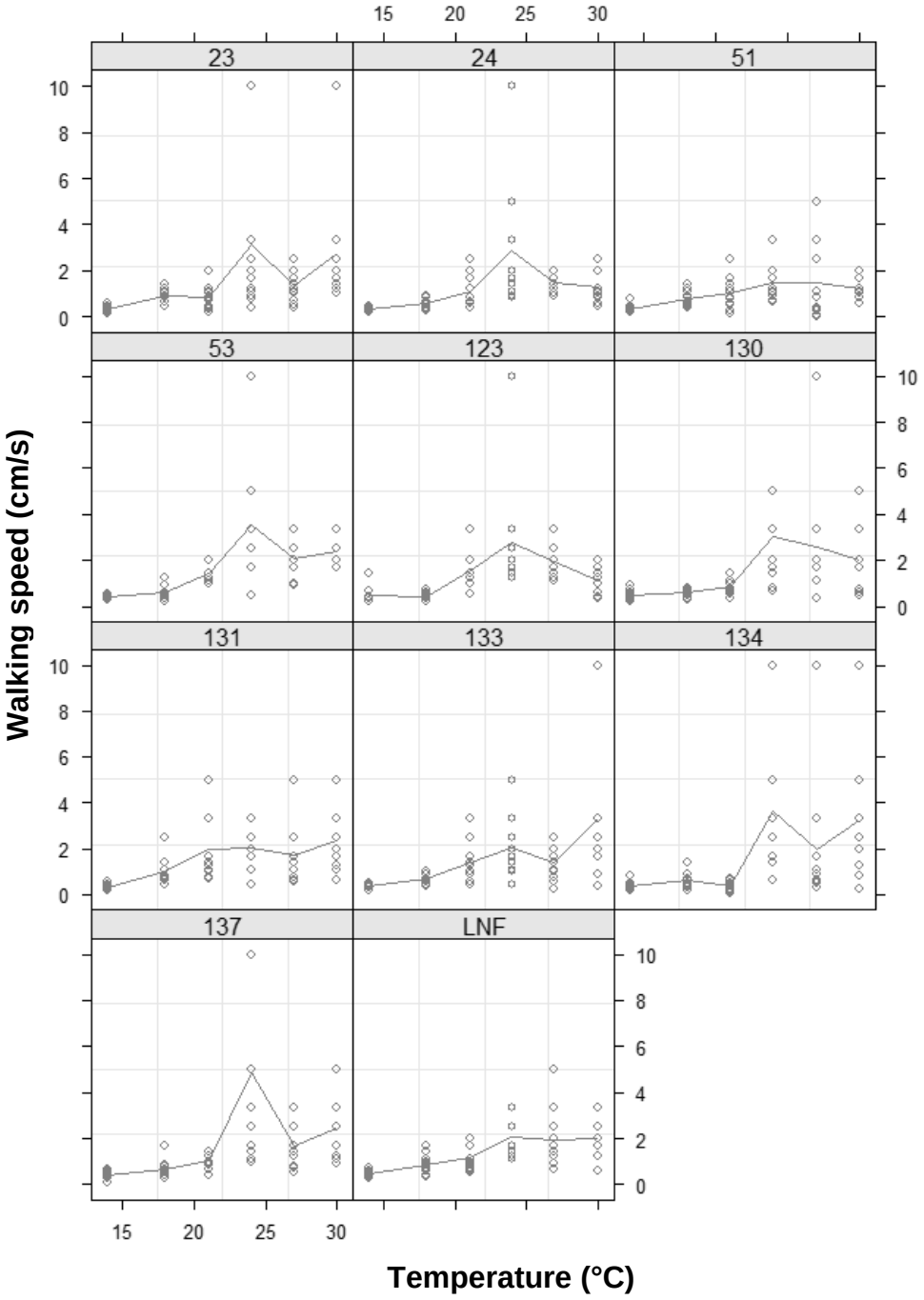

**Appendix 5.** Pupal development time for eleven strains of *D. melanogaster* sampled from across the globe at temperatures from 14 – 30 °C. Diamonds represent the mean development time of the pupae that eclosed from each vial. Strain numbers are reported at the top of each panel. The line is the mean response for each strain at each temperature.



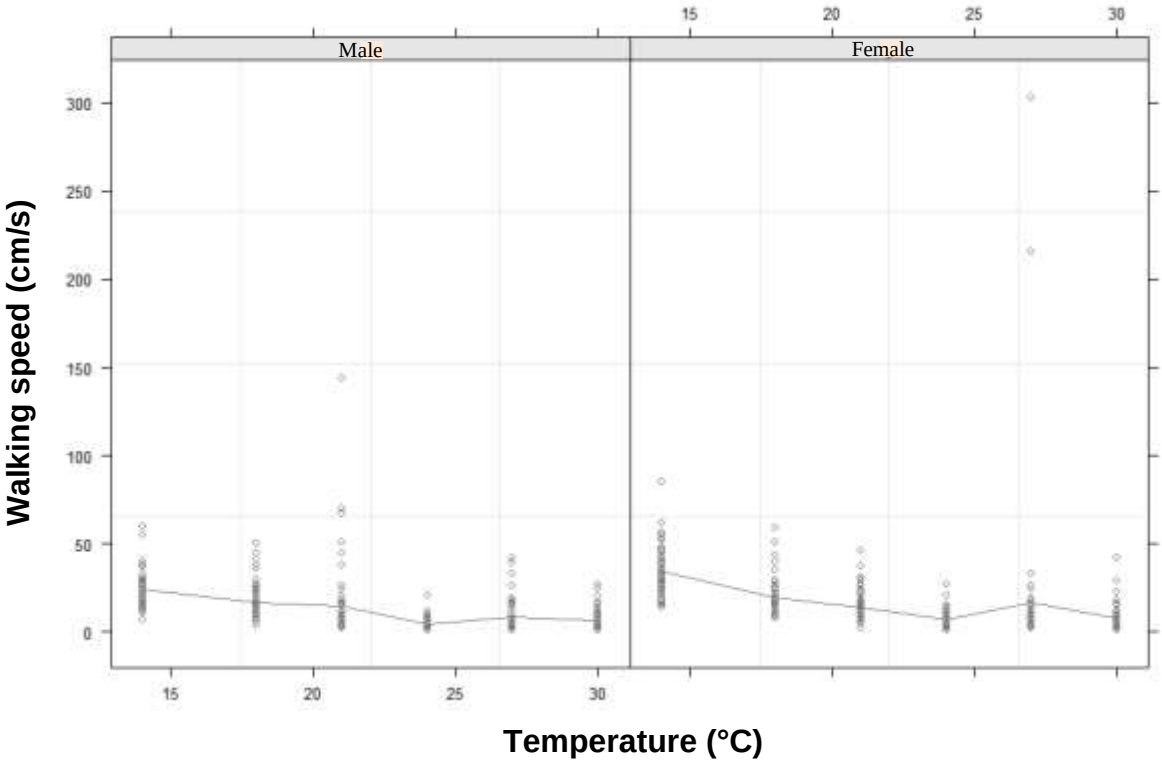

**Appendix 6.** Proportion of males courting during mating assays for eleven strains of *D. melanogaster* sampled from across the globe at temperatures from 6 – 36 °C. Diamonds represent the presence or absence of courtship in one mating assay. Strain numbers are reported at the top of each panel. The line is the mean response for each strain at each temperature.



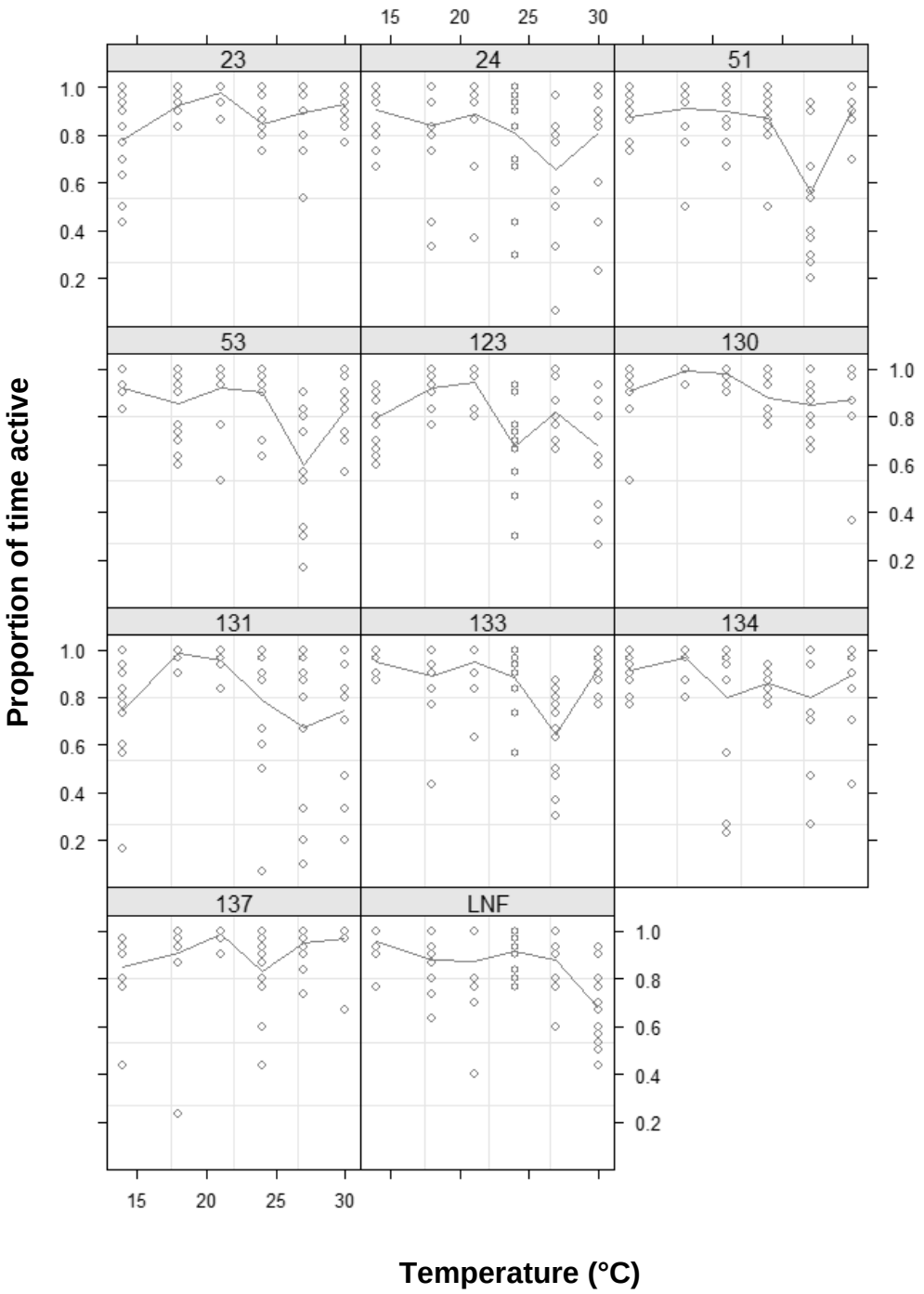

**Appendix 7.** Proportion of pairs copulating during mating assays for eleven strains of *D. melanogaster* sampled from across the globe at temperatures from 6 – 33 °C. Diamonds represent the presence or absence of copulation in one mating assay. Strain numbers are reported at the top of each panel. The line is the mean response for each strain at each temperature.




**Appendix 8.** The walking speed for eleven strains of *D. melanogaster* sampled from across the globe at temperatures from 14 – 30 °C. Diamonds represent the speed of a fly walking a distance of 10 cm. Strain numbers are reported at the top of each panel. The line is the mean response for each strain at each temperature.




**Appendix 9.** The walking speed of *D. melanogaster* sampled from across the globe at temperatures from 14 – 30 °C, separated by sex. Diamonds represent the speed of a fly walking a distance of 10 cm. Sex is reported at the top of each panel. The line is the mean response for each strain at each temperature.



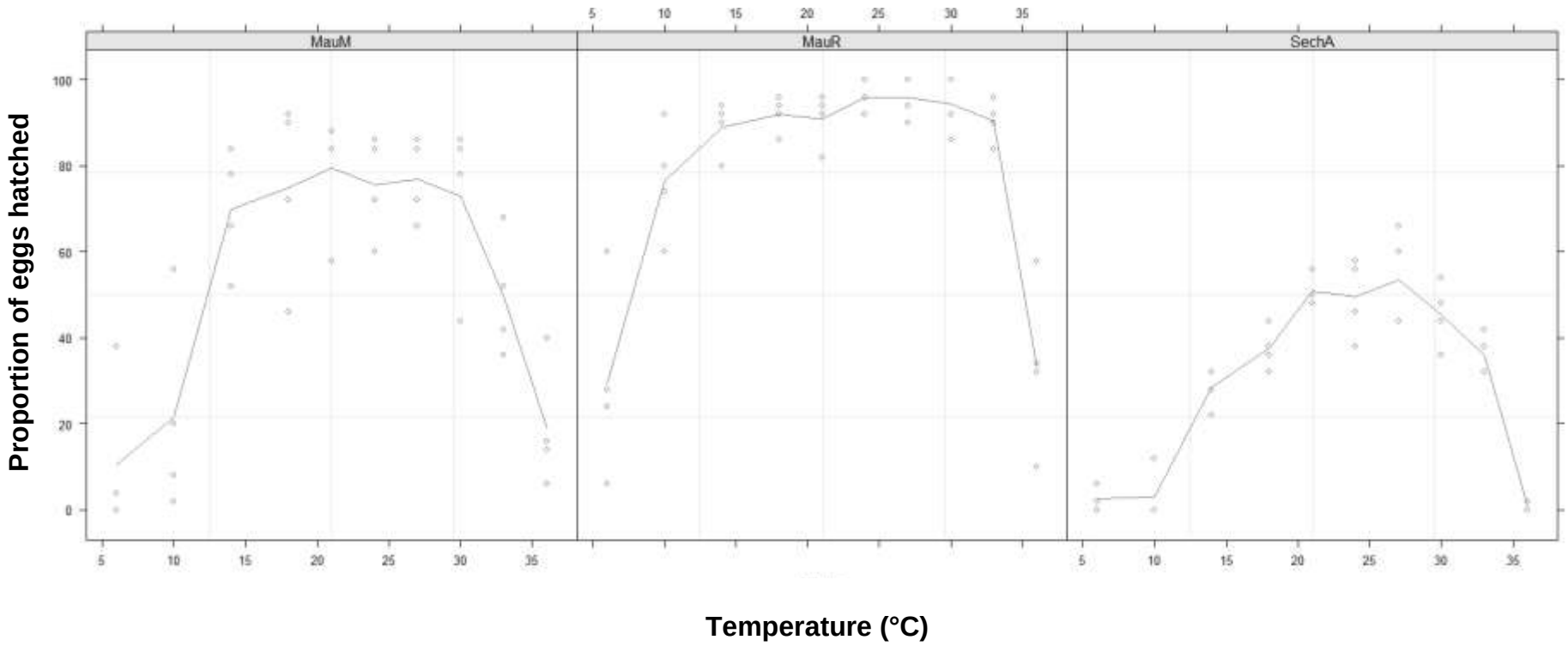

**Appendix 10.** The proportion of time active during 30 s for eleven strains of *D. melanogaster* sampled from across the globe at temperatures from 14 – 30 °C. Diamonds represent the movement of one fly during 30 s. Strain numbers are reported at the top of each panel. The line is the mean response for each strain at each temperature.

**Appendix 11.** Reanalysis of the data from Austin & Moehring (2013).

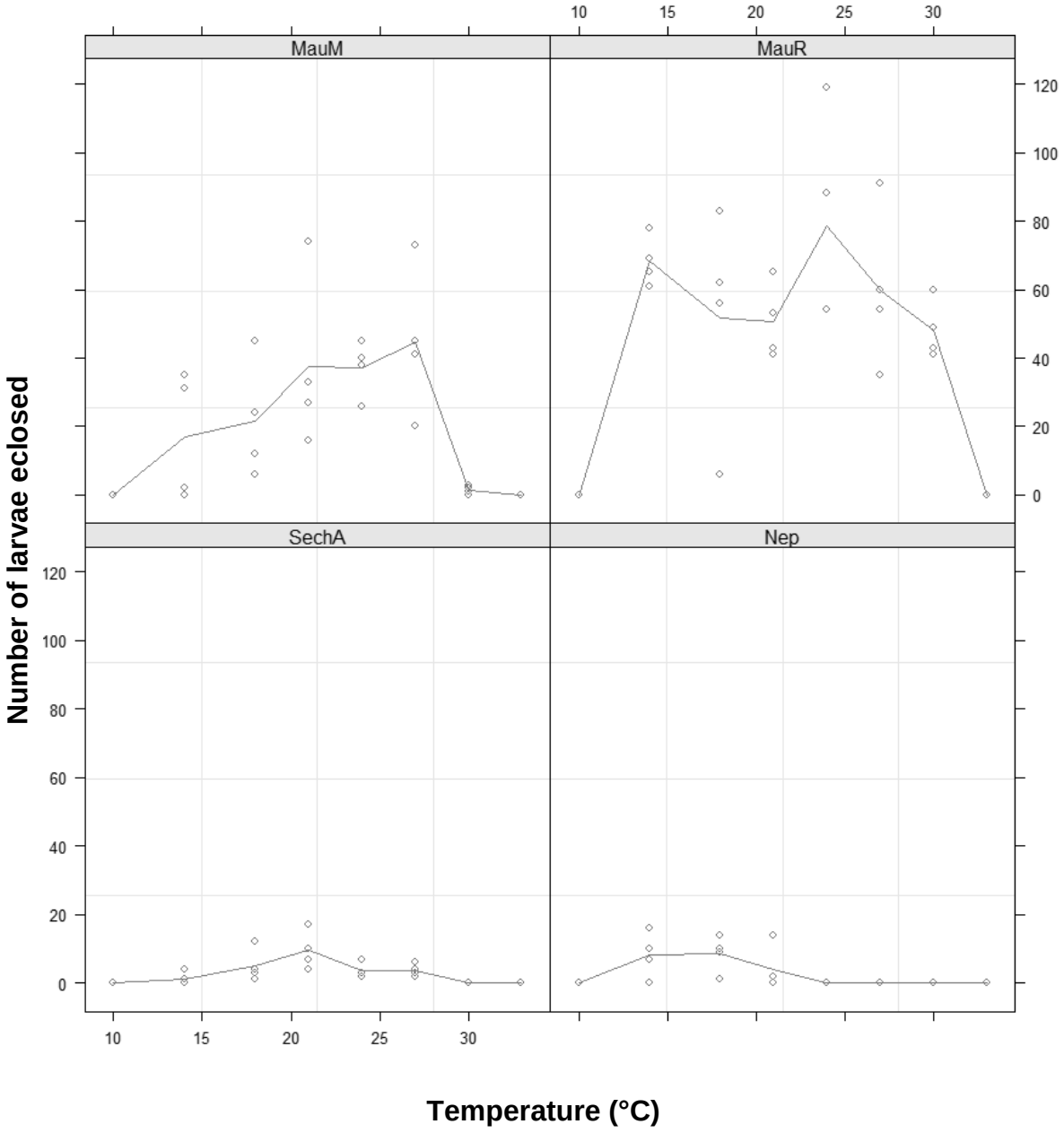
Additional analysis of the data from Austin and Moehring (2013), using the methods that I used in Chapter 2 found additional patterns that were not detected during the statistical analysis of the original published paper. These new results show that there is genetic variation in the way that populations of *D. simulans* respond to experimental temperatures. Significant experimental temperature by strain interactions were detected at the larval eclosion stage ($\beta$ = 0.065, $\chi^2$ = 51.521, df = 10, $P$ < 0.001), and at the adult stage for male courtship ($\beta$ = 0.065, $\chi^2$ = 20.423, df = 10, $P$ < 0.05) and copulation behaviours ($\beta$ = 0.065, $\chi^2$ = 27.907, df = 10, $P$ < 0.01). However, after reanalysis with strain categories replaced with the warmest and coldest monthly temperatures, the only model for a life history trait for *D. simulans* that retains a strain by experimental temperature is at the larval stage ($\beta$ = 0.065, $\chi^2$ = 20.424, df = 1, $P$ < 0.001), where all other interactions of each life history traits were not included in the minimally-adequate model. This additional analysis suggests that there is genetic variation among populations of *D. simulans* in their response to experimental temperatures at the larval and adult stages for reproductive behaviours, which is identical to the response seen in *D. melanogaster*. However, after reanalysis with climatic data included in the model, only the larval stage shows a response among populations that is correlated with local climatic conditions. Therefore, there might be local adaptation to temperature occurring for the larval stage of *D. simulans*, but not at the egg or adult stage (Kawecki and Ebert 2004).

The consistent genetic variation in response to temperature of the male and female flies from these populations of *D. simulans* might reflect genetic drift among the populations tested, or potentially some degree of laboratory adaptation, as many of these stocks have been reared in a laboratory environment for decades, or that the climatic measures used in might not be a good reflection of what the selective pressures are in the natural environment of each population (Kawecki and Ebert 2004; Austin and Moehring 2013). However, given that some lines were collected soon before the experiments began, the variation in response that was detected might reflect what would be observed in natural populations. If these results are representative of the natural populations of *D. simulans*, then the species as a whole might not be as locally adapted to its environment as *D.*

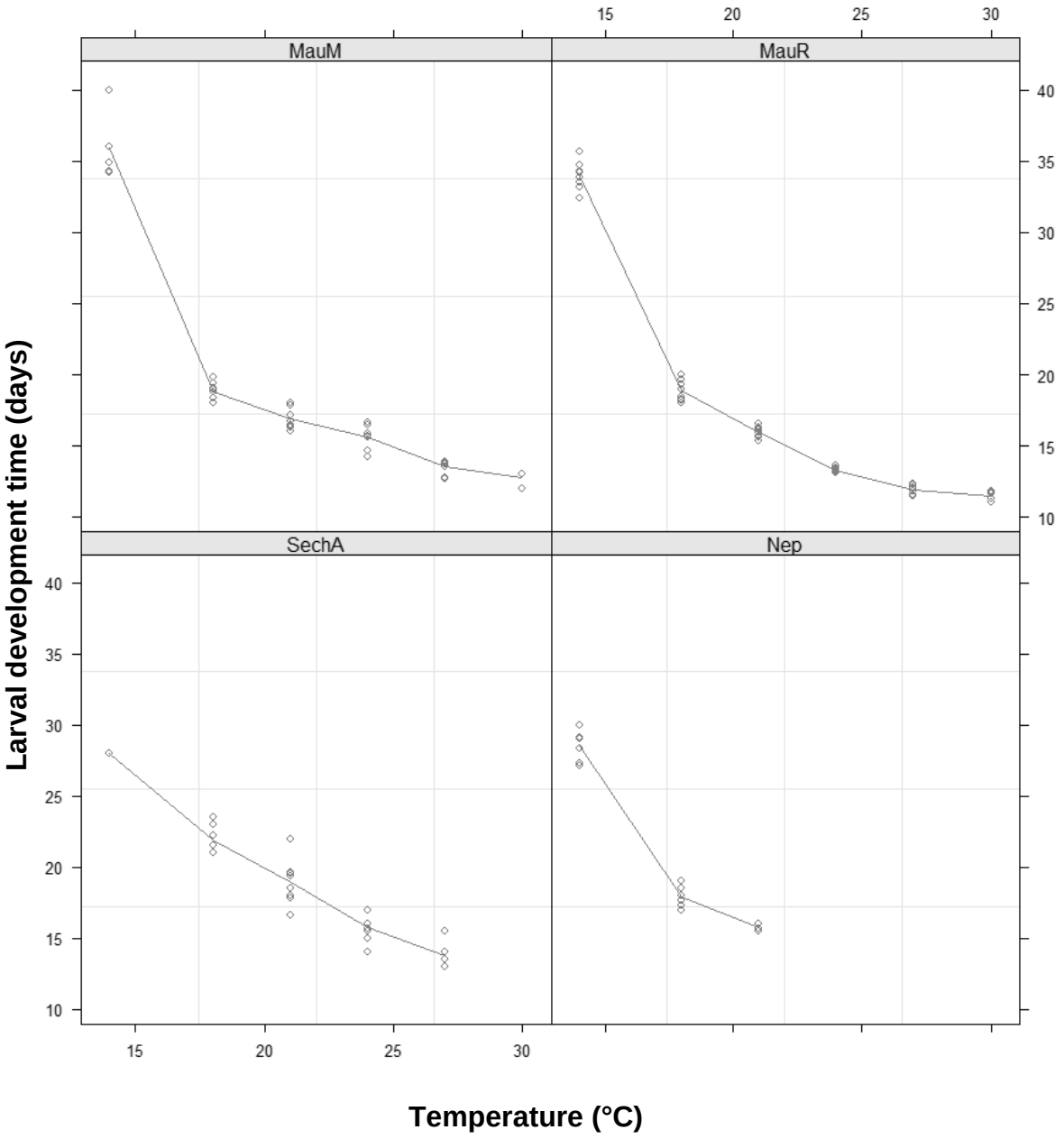
*melanogaster* flies are to their native environments (Kawecki and Ebert 2004). Therefore, the results for the larval stage presented in Austin and Moehring (2013) are different, which reflects that climatic conditions are important to include in models when studying thermal adaptation.



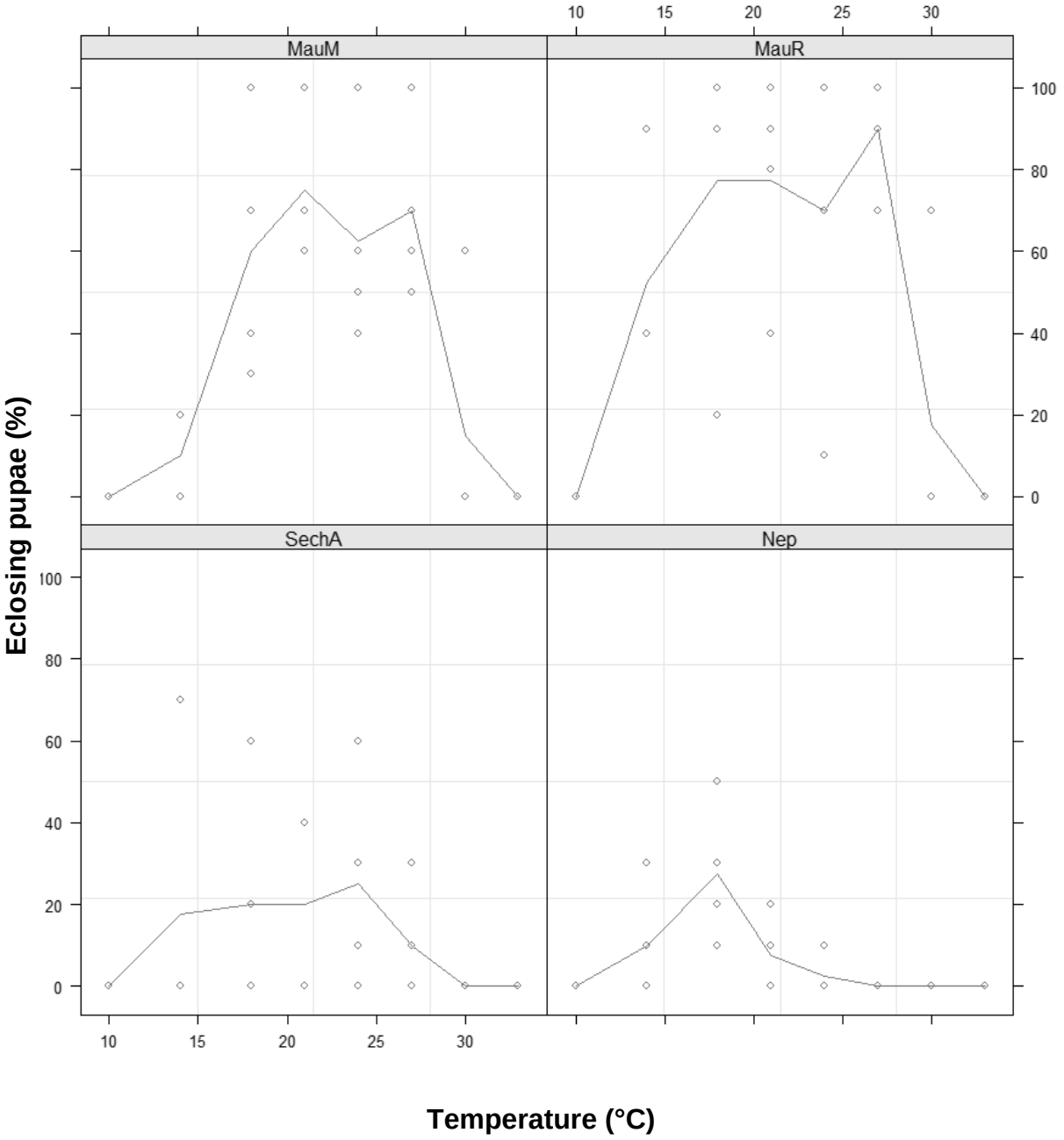

**Appendix 12.** Proportion of eggs hatched for *Drosophila mauritiana* and *D. sechellia* at temperatures from 6 – 36 °C. Diamonds represent the proportion of eggs that hatched out of fifty eggs. Strain names are reported at the top of each panel. The line is the mean response for each strain at each temperature.



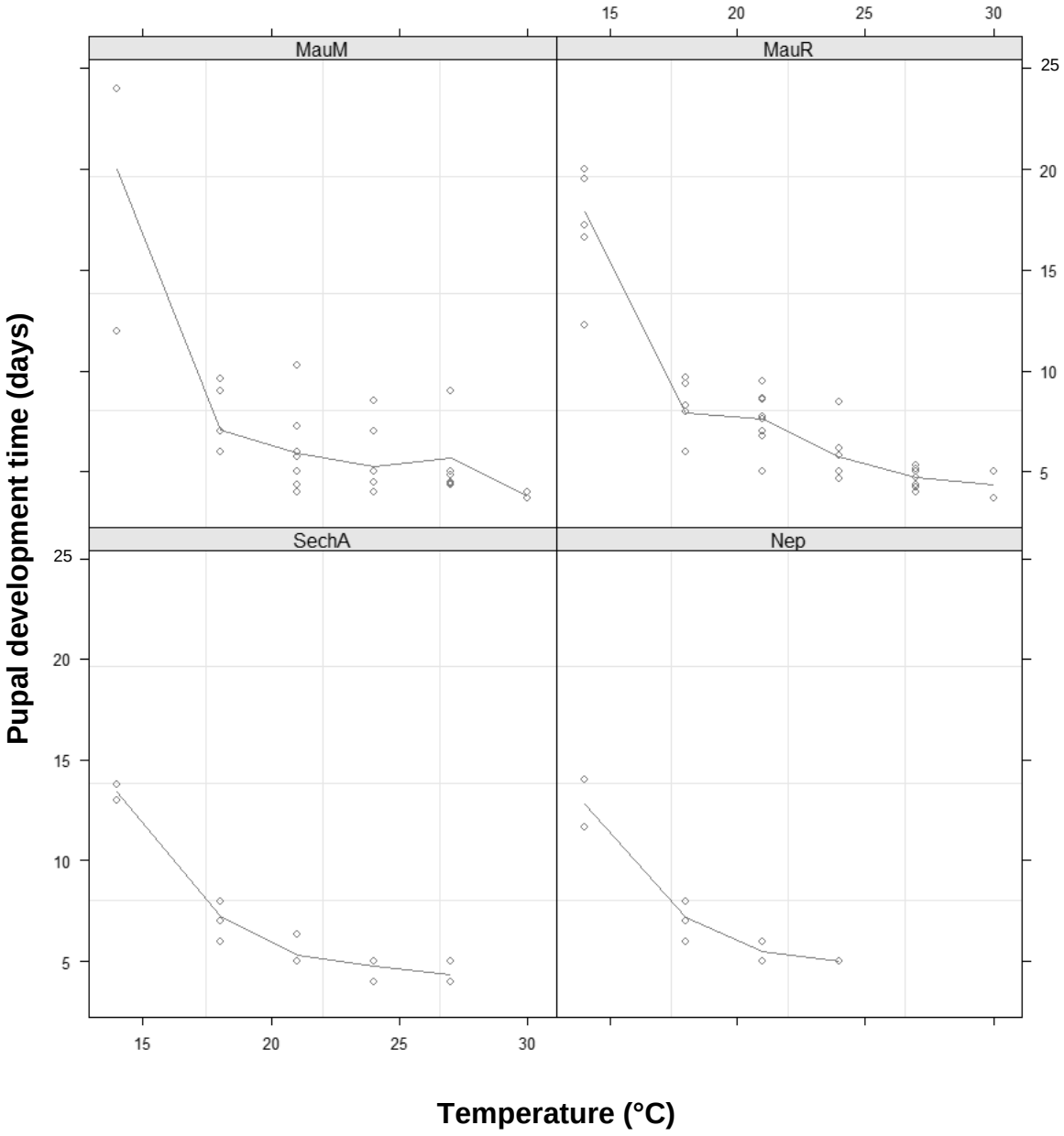

**Appendix 13.** Number of larvae eclosed for *Drosophila mauritiana*, *D. sechellia*, and *D. nepalensis* at temperatures from 6 – 36 °C. Diamonds represent the number of larvae that eclosed from each vial. Strain names are reported at the top of each panel. The line is the mean response for each strain at each temperature.



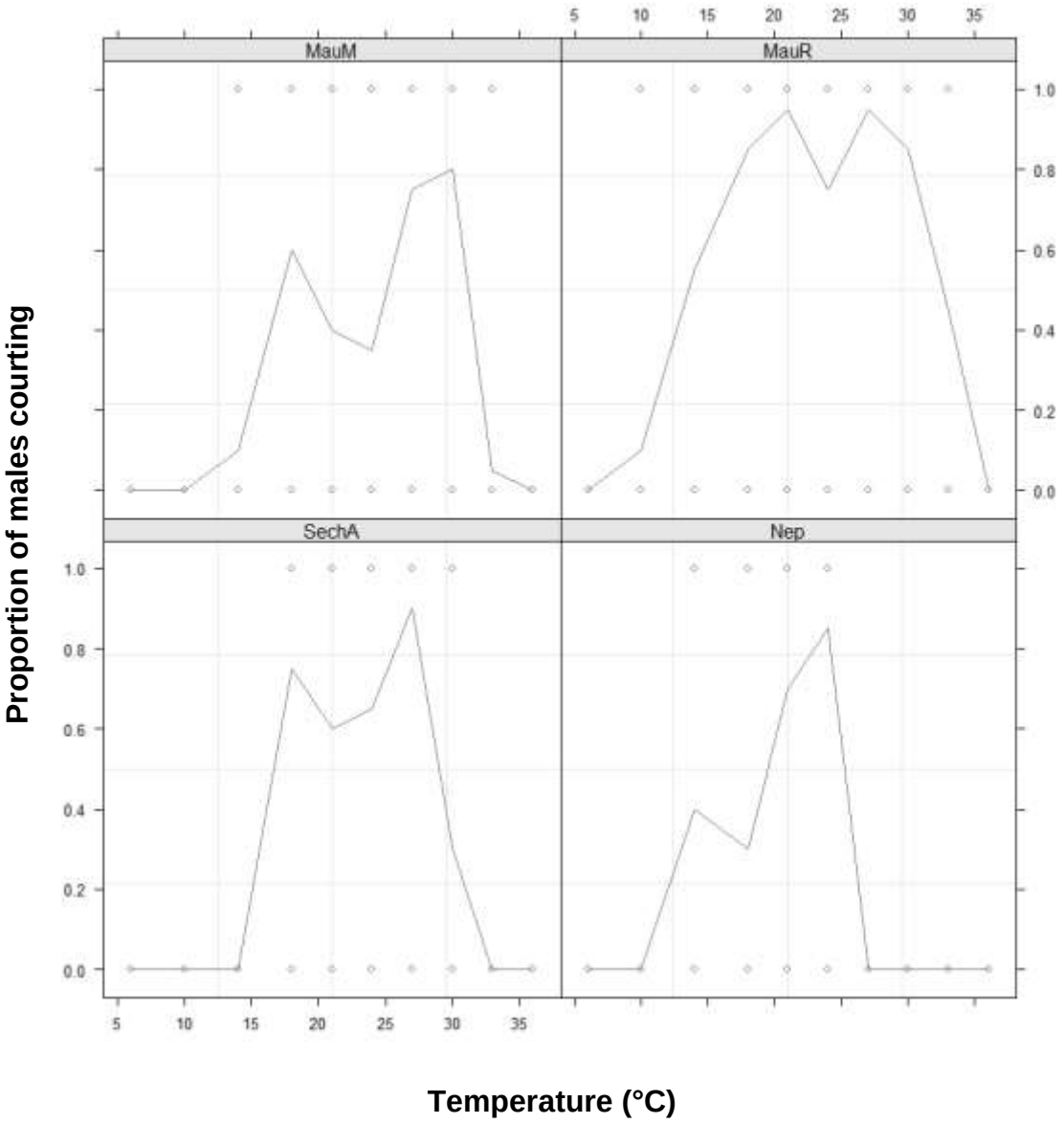

**Appendix 14.** Larval development time for *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* at temperatures from 14 – 30 °C. Diamonds represent the mean development time of the larvae that eclosed from each vial. Strain names are reported at the top of each panel. The line is the mean response for each strain at each temperature.



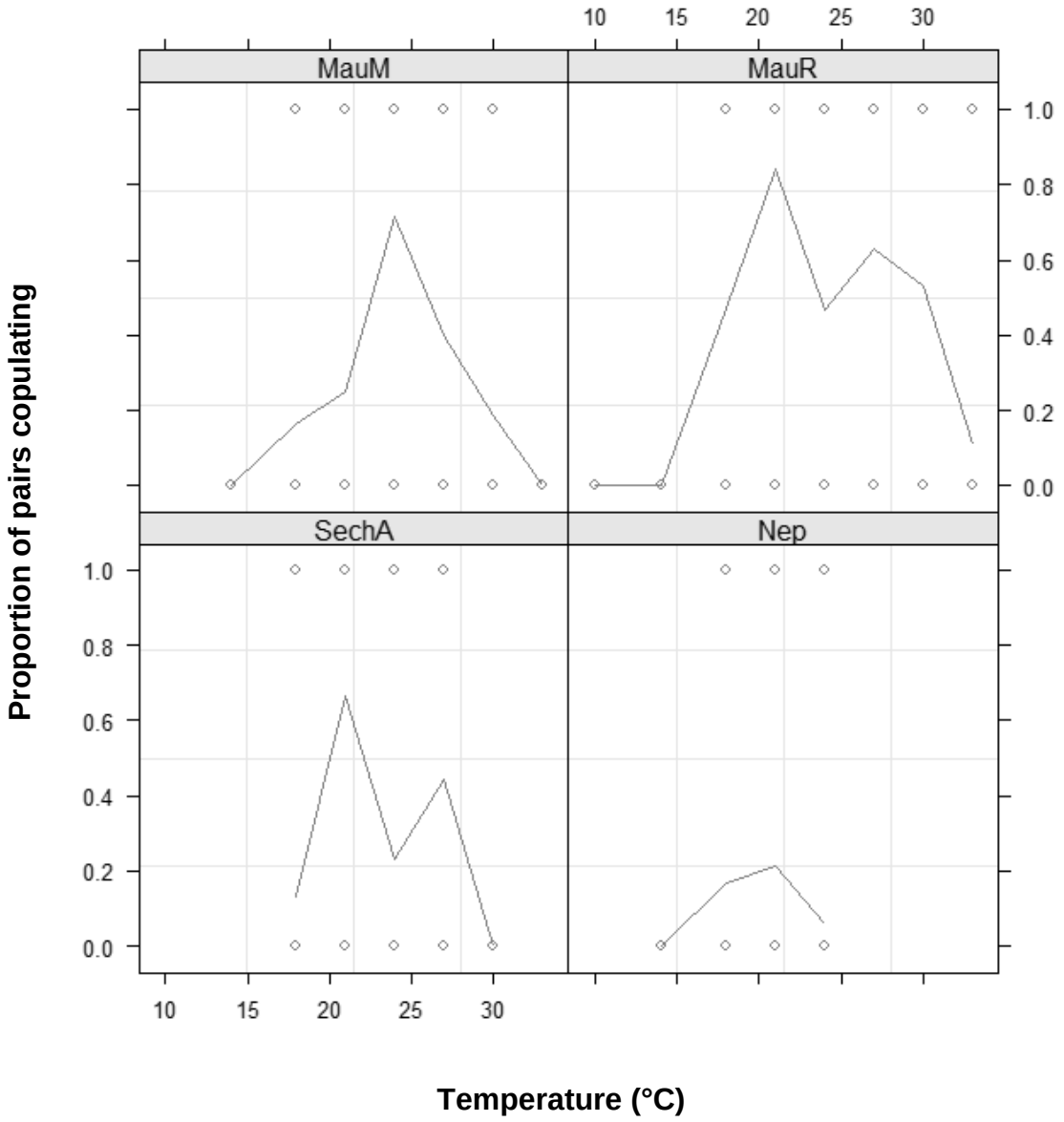

**Appendix 15.** Percent eclosion of pupae for *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* at temperatures from 6 – 36 °C. Diamonds represent the portion of pupae that eclose from each vial out of ten initial third-instar larvae. Strain names are reported at the top of each panel. The line is the mean response for each strain at each temperature.



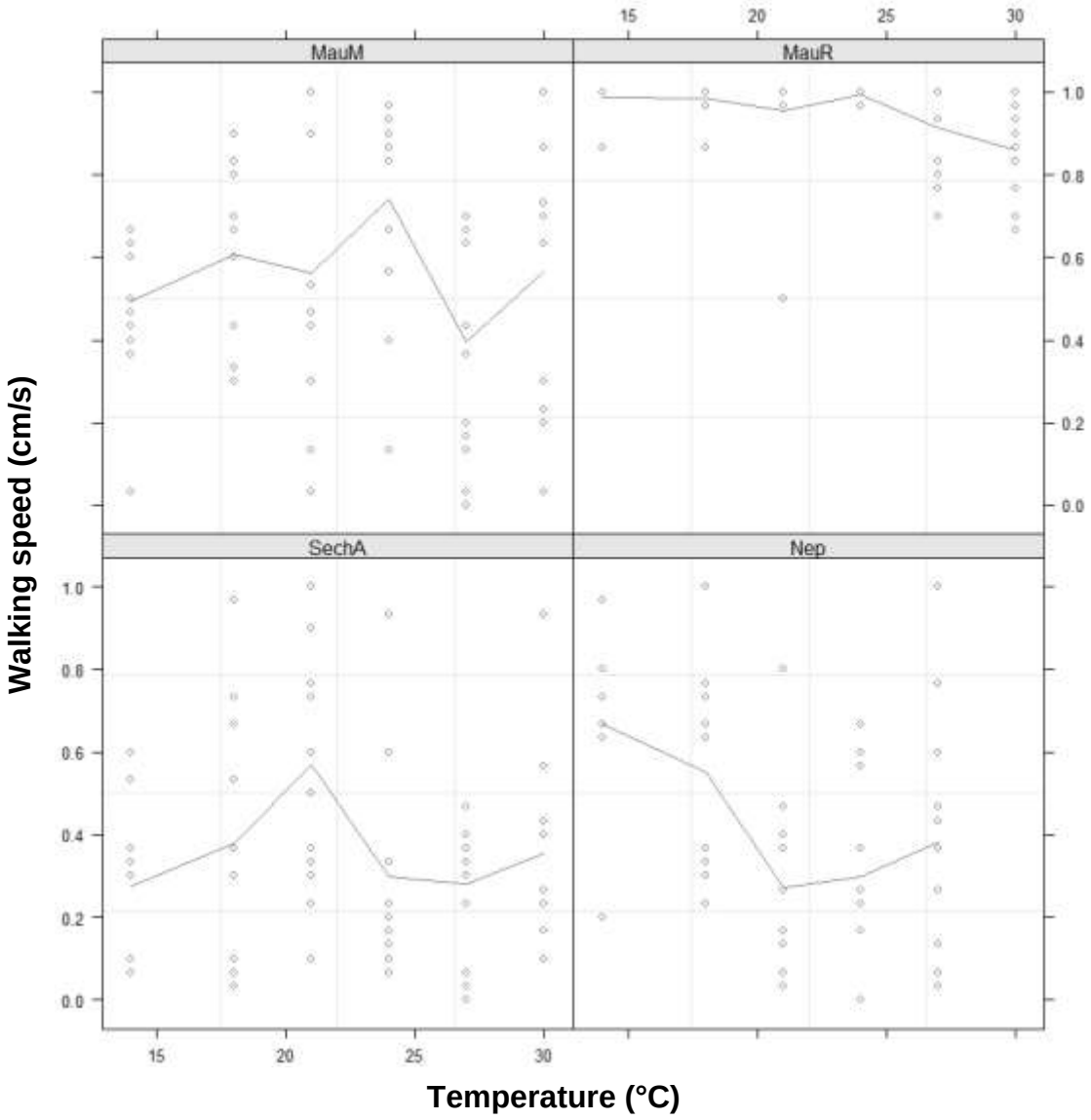

**Appendix 16.** Pupal development time for *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* at temperatures from 14 – 30 °C. Diamonds represent the mean development time of the pupae that eclosed from each vial. Strain names are reported at the top of each panel. The line is the mean response for each strain at each temperature.



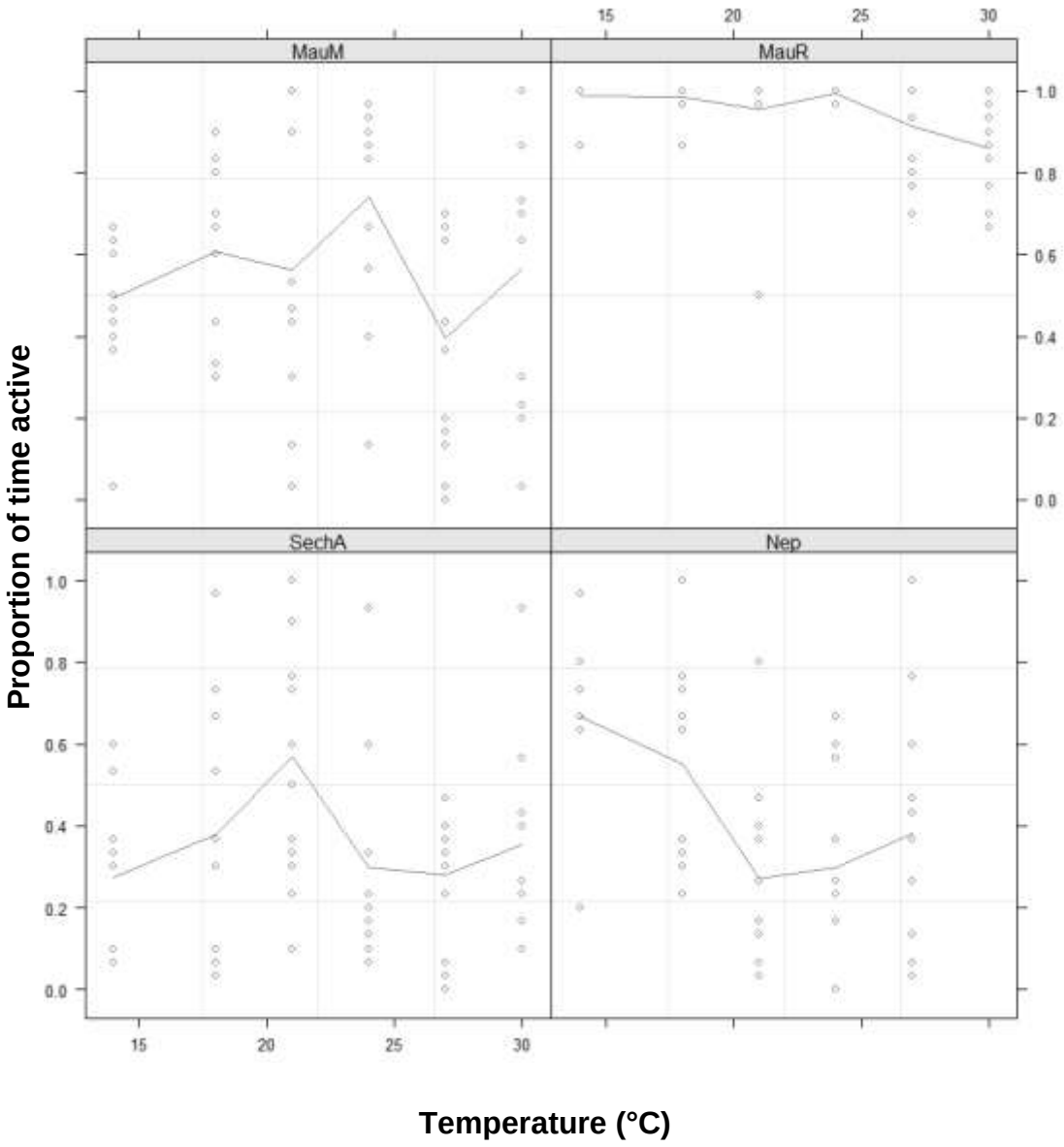

**Appendix 17.** Proportion of males courting during mating assays for *Drosophila mauritiana*, *D. sechellia*, and *D. nepalensis* at temperatures from 6 – 36 °C. Diamonds represent the presence or absence of courtship in one mating assay. Strain names are reported at the top of each panel. The line is the mean response for each strain at each temperature.




**Appendix 18.** Proportion of pairs copulating during mating assays for *Drosophila mauritiana*, *D. sechellia*, and *D. nepalensis* at temperatures from 10 – 33 °C. Diamonds represent the presence or absence of copulation in one mating assay. Strain names are reported at the top of each panel. The line is the mean response for each strain at each temperature.




**Appendix 19.** The walking speed for *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* at temperatures from 14 – 30 °C. Diamonds represent the speed of a fly walking a distance of 10 cm. Strain names are reported at the top of each panel. The line is the mean response for each strain at each temperature.




**Appendix 20.** The proportion of time activity during 30 s for *Drosophila mauritiana, D. sechellia,* and *D. nepalensis* at temperatures from 14 – 30 °C. Diamonds represent the movement of one fly during 30 s. Strain names are reported at the top of each panel. The line is the mean response for each strain at each temperature.

# Curriculum Vitae

**Name:** Christopher J. Austin

**Post-secondary Education and Degrees:**

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| | |
|---|---|
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| 2011 | David M. Scott Scholarship |
| 2011 | UWO Gold Medal |
| 2012 | GTA Academic Achievement Scholarship |
| 2012 | Graduate Research Travel Award (ESC) |
| 2012-2013 | Ontario Graduate Scholarship |
| 2013 | PSAC610 Academic Achievement Scholarship |
| 2013 | Graduate Thesis Research Award (UWO) |

**Related Work Experience**

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The University of Western Ontario
2011-2013

**Publications:**

Austin, CJ & Moehring, AJ. (2013). Optimal temperature range of a plastic species, *Drosophila simulans*. Journal of Animal Ecology. 82:663-672.

Austin, CJ, Guglielmo, CG & Moehring, AJ. (Submitted) A direct test of the effects of changing atmospheric pressure on the mating behavior of *Drosophila melanogaster*. Evolutionary Ecology.

**Presentations:**

Austin, CJ & Moehring, AJ. (2011) Optimum developmental temperature as an indicator of adaptation in *Drosophila*. WURJHNS Undergraduate Research Forum. London, ON, Canada.

Austin, CJ & Moehring, AJ. (2011) Optimum developmental temperature as an indicator of adaptation in *Drosophila*. Toronto Entomologists' Association Symposium. Toronto, ON, Canada.

Austin, CJ & Moehring, AJ. (2011) Optimum developmental temperature as an indicator of adaptation in *Drosophila*. Ontario Biology Day. Waterloo, ON, Canada.

Austin, CJ & Moehring, AJ. (2012) Do some like it hot? Thermal Adaptation in *Drosophila*. Biology Graduate Research Forum. London, ON, Canada.

Austin, CJ & Moehring, AJ. (2012) Do some like it hot? Thermal Adaptation in *Drosophila*. Evolution (ASN, SSE & CSEE). Ottawa, ON, Canada.

Austin, CJ & Moehring, AJ. (2012) Do some like it hot? Thermal Adaptation in *Drosophila*. $OE^3C$. Hamilton, ON, Canada.

Austin, CJ & Moehring, AJ. (2012) Do some like it hot? Thermal Adaptation in *Drosophila*. Three Minute Thesis. London, ON, Canada.

Austin, CJ & Moehring, AJ. (2012) Do some like it hot? Thermal Adaptation in *Drosophila*. Toronto Entomologists' Association Symposium. Toronto, ON, Canada.

Austin, CJ, Guglielmo, CG & Moehring, AJ. (2013) If you can't take the pressure, stay out of the bedroom. A direct test of atmospheric pressure shifts on behaviour. Western Research Forum. London, ON, Canada.

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Austin, CJ, Guglielmo, CG & Moehring, AJ. (2013) If you can't take the pressure, stay out of the bedroom. A direct test of atmospheric pressure shifts on behaviour. Canadian Society of Ecology & Evolution. Kelowna, BC, Canada.

Austin, CJ, Guglielmo, CG & Moehring, AJ. (2013) If you can't take the pressure, stay out of the bedroom. A direct test of atmospheric pressure shifts on behaviour. $OE^3C$. London, ON, Canada.

Austin, CJ, Guglielmo, CG & Moehring, AJ. (2013) If you can't take the pressure, stay out of the bedroom. A direct test of atmospheric pressure shifts on behaviour. Evolution (ASN & SSE). Snowbird, UT, USA.

Austin, CJ & Moehring, AJ. (2013) Do some like it hot? Thermal Adaptation in *Drosophila* – Lightning Talk. Evolution (ASN & SSE). Snowbird, UT, USA.