Local adaptation in climate tolerance at a small geographic scale contrasts with broad latitudinal patterns

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Abstract

If environmental heterogeneity at small geographic scales has a similar capacity to generate adaptive genetic variation to that found across broad latitudinal gradients, then we currently underestimate adaptive capacity to rapid global change. To contrast adaptive genetic variation across geography, we quantified population variation in tolerance traits and their thermal plasticity for five populations of *Drosophila melanogaster* from a 3000-km latitudinal gradient across east coast Australia, with eight populations from an environmentally heterogeneous 600×300km area within Victoria. Surprisingly, population variation in stress tolerance within Victoria was comparable to that across latitude. Consistent with local adaptation, heat and desiccation tolerance were greater for populations from warmer and drier environments, and populations from more predictable environments showed greater thermal plasticity. Adaptation to environmental heterogeneity at smaller geographic scales can therefore contribute more to adaptive capacity than originally thought. However, patterns of adaptation often changed across geography and treatments, which makes predicting responses to global change challenging.

Keywords: desiccation, *Drosophila*, geographic variation, heat tolerance, heterogeneous landscape, intraspecific variation, latitudinal cline, local adaptation, plasticity, thermal tolerance

Introduction

Ongoing global change is creating increasingly stressful and unpredictable environments, which is fragmenting available habitat for mobile species, and increasing the vulnerability of long-lived species and those with low dispersal (Parmesan & Yohe 2003; Parmesan 2006). It is therefore likely that many organisms will need to adapt *in situ* if they are to persist. Rapid adaptation to global change will be more likely if gene flow readily moves adaptive genetic variation across an environmentally heterogeneous landscape (Sexton *et al.* 2011; Sgrò *et al.* 2011; Willi *et al.* 2022; Sexton *et al.* 2024). Predicting the vulnerability of species then relies on understanding how much adaptive genetic variation is available and how it is distributed among populations across landscapes (Razgour *et al.* 2019; Urban *et al.* 2024). However, we still know little about the scale at which environmental heterogeneity generates and maintains adaptive genetic variation, which limits our ability to predict the adaptive capacity of species facing rapid global change (Byers 2005; Whitlock 2015; Alvarado *et al.* 2022; Meek *et al.* 2023).

The geographic scale at which adaptation arises across a landscape is rarely considered when assessing vulnerability to global change (Bennett *et al.* 2019). Understanding how environmental heterogeneity generates adaptation across geography reveals how gene flow and local selection pressures interact to determine the extent of adaptive genetic differentiation (Slatkin 1987; García-Ramos & Kirkpatrick 1997; Richardson *et al.* 2014; DeMarche *et al.* 2019; Booker 2024). Such knowledge is crucial for predicting how species will respond to ongoing global change. Local adaptation at smaller geographical scales can facilitate more rapid movement of adaptive alleles across a metapopulation, which increases the adaptive capacity of populations (Meek *et al.* 2023). While patterns of adaptation across broad environmental gradients are common (e.g., Huey *et al.* 2000; Etterson 2004; Ren *et al.* 2020; Aspinwall *et al.* 2023), we critically lack tests of whether these same patterns of adaptation emerge at small geographic scales.

Studies of climatic adaptation typically sample broadly across latitude covering thousands of kilometres. Latitudinal clines in stress tolerance traits reflect adaptive genetic variation because genetic differences are shaped by local climatic selection that changes along the latitudinal gradient (Huey *et al.* 2000; Hoffmann *et al.* 2002; Etterson 2004; Trotta *et al.* 2006; Sgrò *et al.* 2010; Lasne *et al.* 2018; Lush *et al.* 2024). However, if local adaptation occurs at smaller geographic scales (e.g., tens or hundreds of kilometres), then adaptive genetic variation could be generated and maintained at scales where gene flow is more likely to move adaptive alleles among populations (Stotz *et al.* 2021; Alvarado *et al.* 2022). While the idea that environmental heterogeneity can generate adaptive genetic variation at different geographic scales has been around for some time (e.g., Cowling & Pressey 2001; Pressey *et al.* 2007; Sgrò *et al.* 2011), few studies have empirically contrasted the extent of adaptation across geographic scales.

Empirical tests of adaptation at multiple geographic scales across a heterogenous landscape are scarce. Hoffmann *et al.* (2001) found little evidence of variation among populations of *Drosophila* c.20–100km apart compared to latitudinal extremes (>2000km). By contrast, subtle differentiation in genes across

69 distinct tidal environments at a local scale (<100km) were associated with temperature in a sea urchin
70 despite little population structure across latitude (Rumberger *et al.* 2025). Moreover, local adaptation
71 across elevation reveals the potential for adaptation across an ecological gradient (Gonzalo-Turpin &
72 Hazard 2009; de Villemereuil *et al.* 2018; Halbritter *et al.* 2018; Tregenza *et al.* 2021). While these
73 studies show that local environmental differences can generate adaptive differentiation, we still do not
74 understand how adaptive capacity differs for populations distributed across environments at a local versus
75 broad geographic scale. We therefore need studies that directly contrast climate adaptation along a broad
76 gradient with populations across a small geographic scale where local adaptation could still arise.

77 In addition to adaptation, plastic changes in climate tolerance traits are also expected to play an important
78 role in helping populations buffer global change (Reed *et al.* 2011; Gunderson & Stillman 2015; Magozzi
79 & Calosi 2015). Environments that are variable and predictable should favour the evolution of greater
80 plasticity (Scheiner 1993; Leung *et al.* 2020), which can lead to populations that differ in plasticity when
81 they adapt to different environments (Lind & Johansson 2007; Murren *et al.* 2014; Matesanz & Ramírez-
82 Valiente 2019; Walter *et al.* 2022). While populations sampled across latitude vary in plasticity for
83 thermal traits that reflect climatic selection for plasticity (Sgrò *et al.* 2010; van Heerwaarden & Sgrò
84 2017), we still lack the ability to connect environmental predictability with the evolution of adaptive
85 plasticity across a heterogeneous landscape.

86 To contrast the extent of adaptive genetic variation in key traits across geographic scales, we focus on
87 geographic variation in populations of the vinegar fly, *Drosophila melanogaster,* in Australia. Since its
88 introduction to the east coast of Australia, c.100 years ago, *D. melanogaster* has developed clines across
89 c.3000km for numerous traits, including thermal tolerance, desiccation and starvation tolerance and body
90 size (reviewed in Hoffmann & Weeks 2007; Lasne *et al.* 2018). Molecular data suggests that population
91 structure is weak along latitude due to some level of gene flow at distances up to 1500km (Gockel *et al.*
92 2001; Kennington *et al.* 2003). The presence of latitudinal clines in ecologically important traits provides
93 strong evidence that adaptive genetic variation is generated across latitude (Huey *et al.* 2000; Hoffmann
94 *et al.* 2002). Such broad patterns of adaptation and the potential for high gene flow means that *D.*
95 *melanogaster* provides a powerful conservative test of whether small-scale geographic variation can
96 generate and maintain adaptive genetic variation.

97 We sampled five populations of *D. melanogaster* across 3000-km in latitude along east coast Australia
98 (18°S–43°S), which we compared to eight populations sampled from a variety of climates within a
99 600km×300km area in Victoria (**Fig. 1a**). Compared to the latitudinal populations, locations sampled in
100 Victoria showed greater variation in mean temperature, both across days (**Fig. 1b**) and within days (**Fig.**
101 **1c**). To ensure our estimates of among-population variation would be conservative, we sampled flies in
102 Autumn, which is after the summer peak population size that has the greatest potential for
103 homogenization of populations via dispersal (Shpak *et al.* 2010; Behrman *et al.* 2015). Using a

manipulative laboratory experiment, we reared larvae from all 13 populations under three temperature treatments and measured wing size and tolerance traits (heat, cold and desiccation) on emergent adults. The data for the latitudinal populations were used previously to show that latitudinal clines changed more across temperature treatments than in response to a low-calorie diet (Walter *et al.* 2025). Here, we test three hypotheses to contrast the extent of adaptive differentiation in climate tolerance across geography: (I) We would detect variation among populations in Victoria for climate tolerance traits, but populations from across latitude would vary to a greater extent. (II) If the among-population variation is adaptive, we would observe associations between climate of origin and tolerance traits for both latitudinal and Victorian populations. (III) If adaptation generates differences in plasticity, then population variation in plasticity would be associated with environmental predictability.

Methods

Collection and maintenance of experimental populations

In March–May 2021, we collected 20–50 field-inseminated female *D. melanogaster* from thirteen locations in Australia using sweep nets over fruit waste. For the five latitudinal populations, we sampled flies at fruit farms in Tasmania, New South Wales, Victoria and Northern Queensland (**Fig. 1a**; **Table S1**). In Victoria, we sampled flies from discarded grape skins at eight wineries from a variety of climates (**Fig. 1a**; **Table S1**). We chose wineries due to the abundance of breeding habitat at the same time of year, allowing us to sample broadly across the state within a short period.

In the lab, we generated isofemale lines by allowing inseminated females to individually lay for 4 days in 40mL vials containing 7mL of standard yeast-potato-dextrose medium (Brewer's yeast 36.4g/L; potato flakes 18.2g/L; dextrose 27.3g/L; agar 6.4g/L, nipagin [10% w/v in ethanol] 10.9mL/L; and propionic acid 4.6mL/L), and leaving the larvae to develop at 25°C on a 12:12 hour light:dark cycle. In the following generation, we checked species identification to prevent contamination from the morphologically similar *D. simulans.* In the third generation, we treated each isofemale line with 0.3mg/mL of tetracycline in the food to remove differences among populations in their endosymbionts, particularly *Wolbachia*, which is more prevalent in tropical populations. After three generations, for each population we combined five males with five virgin females from each isofemale line (19–60 lines per population, mean=44.6; **Table S1**) into a mass bred population that we maintained in five 300mL bottles containing 62.5mL of standard food. Each generation, we collected adults and kept them in bottles until they reached peak fertility (c.4 days), after which they were allowed to lay 250–300 eggs in each of the five bottles. After laying, we removed the adults, added cards for pupation and left the larvae to develop.

We maintained the 13 populations at 25°C for 12 generations (c.9 months) to ensure that genotypes were sufficiently mixed, and to avoid conducting experiments during COVID-19 lockdowns in Melbourne.

Manipulative experiment

In January 2022, we conducted a manipulative laboratory experiment with the 13 mass-bred populations to test for variation among populations in cold, hot and desiccation tolerance in response to temperature treatments. We picked eggs from all 13 populations into three temperatures (13, 25 and 29°C). 25°C represents control conditions at which the populations were maintained, and 13 and 29°C represent the cold and hot margins that larvae in natural populations are often exposed to in winter and summer, respectively. For each population, we placed c.200 adult flies in each of two laying pots with a thin (c.3mm) layer of food with double agar and let them lay for 6 hours. We then transferred 20 eggs into each vial (n=36–42 vials/population/treatment; **Table S2**) containing 7mL of standard food and left larvae to develop at their treatment temperatures. To manage the replication, we split the experiment into two blocks so that adults emerged 21–28 January (block 1) and 1–8 March (block 2).

For each block, we conducted 2–3 rounds of egg-picks for each treatment split over 4–5 days to maximise the overlap in eclosion across treatments (n=14–21 vials/population/treatment/block). We timed the egg picks for the different treatments according to pilot experiments so that adult flies would emerge simultaneously. Overall, we used 1456 vials in total (n=455–546 vials per treatment).

During eclosion, adults from each treatment were collected in separate vials each day so that flies would be the same age across treatments. Flies were then kept at their experimental treatments until they reached maturity and had mated. Pilot experiments suggested flies kept at 13°C would not mature, and so they were kept at 16°C to mature in time for the experiment.

Stress tolerance assays and body size measurements

We measured females as their body size and stress tolerance are more sensitive to variation in nutrition and temperature (Kutz *et al.* 2019; Chakraborty *et al.* 2020), and because sex-specific adaptation was not our focus. We collected females under light CO_2 anaesthesia, let them recover for 48 hours and then used established methods to measure heat, cold, or desiccation tolerance, or body size (Hoffmann *et al.* 2002; Lasne *et al.* 2018). We measured 30 females per assay. To assay heat tolerance, we measured heat knockdown by putting individual females into sealed 5mL glass vials, submerging them in a 39°C water bath and measuring the time for each female to cease moving. To assay cold tolerance, we measured chill-coma recovery by placing individual females into sealed 1.5mL plastic tubes that we submerged in a 0°C water bath for 4 hours and measured the time they took to regain movement. To measure desiccation tolerance, we put individual females in unsealed 5mL vials placed in sealed glass tanks containing silica gel that maintained relative humidity at <5%. We then recorded the time it took for each fly to cease moving. To measure wing size, we removed the right wing of 30 females, mounted them on a microscope

slide and photographed them using a Leica M60 stereo microscope (Leica, Heerbrugg, Switzerland). We then used *imageJ* to quantify 10 vein intersection landmarks (**Fig. S1**), which we used to estimate centroid size.

Statistical analyses

We used *R* (v.4.3.2) statistical software for all analyses (R Core Team 2024). With *glmmTMB* (Brooks *et al.* 2017), we used linear mixed models to test how each trait varied across the 13 populations, and how such variation was associated with the environment of origin. We checked model assumptions using *DHARMa* (Hartig 2022).

Hypothesis I: Testing for among-population variation in tolerance traits and body size

To test for significant variation among populations in response to treatment, we included population and temperature as fixed effects, and tested for a significant population×temperature interaction using type-III ANOVA. We performed the analysis on latitudinal and Victorian populations separately. Replicate run nested within block was the only random effect.

To compare differences in plasticity, we extracted the population marginal means from the models above using *emmeans* (Lenth 2019), and then calculated plasticity using: $plasticity = \frac{(treatment\ mean - control\ mean)}{control\ mean}$ (Valladares *et al.* 2006), which represents plasticity as the proportional change in phenotype from control conditions (25°C).

Hypotheses II: Testing whether among-population variation is adaptive

We tested for associations between climate of origin and stress tolerance traits. For all 13 sampling locations, we first extracted fine-scale rainfall (daily) and temperature (hourly) data using *Nichemapr* (Kearney & Porter 2020) for the four months preceding population sampling. We calculated daily mean temperature and then quantified predictability of temperature by calculating autocorrelation across days using the *acf* function. We then tested whether climate tolerance traits and wing size were associated with environment of origin using the linear mixed model

$$y_{ijklm} = O \times G_i \times T_j + p_{k(ij)} + r_l + e_{m(ijkl)} \qquad (1)$$

where the three-way interaction of the fixed effects ($O \times G_i \times T_j$) includes environment of origin (O) measured at the population locations (continuous variable), the geographical comparison (G_i; Victoria vs latitude) and treatment temperature (T_j). Random effects included the *k*th population nested within treatment temperature and geography ($p_{k(ij)}$) to avoid pseudoreplication, and the *l*th replicate run within experimental block (r_l). $e_{m(ijkl)}$ represents the residual, and y_{ijklm} the response variable. We applied equation 1 to each tolerance trait and body size separately. We included mean temperature as the environmental covariate (O) for analysing thermal tolerance and body size, and mean daily rainfall when analysing desiccation tolerance.

A significant slope for O (effect of environment) provides evidence that environment of origin is associated with that particular trait. Where the two-way interaction between environment of origin and geography is significant suggests that latitudinal and Victorian populations differ in their association between tolerance traits (or body size) and environment of origin, and therefore show different patterns of adaptation. A significant three-way interaction then provides evidence that associations between environment of origin and the tolerance trait (or body size) is different for latitudinal and Victorian populations, and this also depends on the treatment temperature.

Finally, to test whether environmental predictability was associated with population variation in thermal plasticity, we applied equation 1 but used estimates of plasticity in heat tolerance as the response variable, and autocorrelation of temperature across days (capturing predictability of temperature) as the environmental covariate.

Results

Hypothesis I: High variation among Victorian populations for tolerance traits

Temperature created significant changes in stress tolerance and body size (**Fig. 2**, **Table 1**). As expected, flies exposed to colder temperatures were larger, more cold tolerant and less heat tolerant. We found significant variation among populations in all tolerance traits and body size (**Table 1**). Body size was the only trait where variation among populations was much greater across latitude compared to Victoria (**Fig. S2**), which was consistent across all treatments (**Fig. 2**). Somewhat surprisingly, this meant that Victorian populations showed similar levels of variation among populations compared to the latitudinal populations for all tolerance traits (**Fig. 2**, **Fig. S2**). These results suggest that environmental heterogeneity at smaller geographic scales can generate substantial among-population variation in tolerance traits.

Statistically significant interactions between population and temperature quantifies how populations vary in response to treatments, and therefore reflects population variation in plasticity. For cold and heat tolerance, both Victorian and latitudinal populations showed significant temperature×population interactions (**Table 1a-b**). Only desiccation tolerance showed no significant population×temperature interactions (**Table 1c**). For wing size, only latitudinal populations showed a significant population×temperature interaction (**Table 1d**). Therefore, Victorian and latitudinal populations both displayed significant variation in thermal plasticity (**Fig. 3**).

Hypothesis II: Population variation is associated with climate of origin

Along the east coast, temperatures were higher and more consistent at lower latitudes. By contrast, Victorian sampling locations had greater variation both in temperature across days (**Fig. 1b**) and within

237 each day (**Fig. 1c**). The Victorian locations therefore experience greater thermal variation compared to
238 latitudinal locations. Rainfall was lower at high latitudes, ranging 193–984mm south–north, while
239 Victoria varied between 130–428mm (**Table S1**). To test whether among-population variation in
240 tolerance traits and body size was adaptive, we quantified associations between climate of origin with the
241 traits measured in the laboratory. Below we summarise the results for each trait.

242 Cold tolerance: We found a significant three-way interaction between temperature of origin (O),
243 geography (G; latitude vs Victoria) and treatment temperature (T; **Fig. 4a**; **Table S3**). While there was no
244 association between temperature of origin and cold tolerance at 13°C, at the warmer temperatures (25°C
245 and 29°C), latitudinal populations from warmer temperatures showed slower cold recovery (reduced cold
246 tolerance), as expected (**Fig. 4a**). By contrast, Victorian populations showed the opposite trend where
247 populations from warmer environments showed higher cold tolerance (**Fig. 4a**), suggesting that they were
248 better at coping with a cold shock after acclimating to warmer conditions.

249 Heat tolerance: We found no significant effect of geography, suggesting that associations between
250 temperature and heat tolerance were consistent for Victorian and latitudinal populations (**Fig. 4b; Table**
251 **S3**). Associations between temperature of origin and heat tolerance changed depending on the treatment
252 temperature, which was supported by a significant temperature of origin × treatment temperature
253 interaction (**Fig. 4b; Table S3**). While we found no strong associations at 13°C, at the warmer (25°C and
254 29°C) treatments, populations from warmer locations showed higher heat tolerance, creating a positive
255 association between temperature of origin and heat tolerance (**Fig. 4b**). We therefore found evidence of
256 local adaptation in warmer treatments, which was consistent for Victorian and latitudinal populations.

257 Wing size: Consistent with latitudinal studies, populations from lower latitudes had smaller wings (**Fig.**
258 **4c**). However, Victorian populations showed a contrasting trend where populations from warmer
259 environments had slightly larger wings (**Fig. 4c**). This result was supported by a significant temperature
260 of origin × geography (latitudinal vs Victoria) interaction (**Fig. 4c**; **Table S3**). Wing size therefore
261 showed contrasting associations with temperature of origin for Victorian versus latitudinal populations,
262 and this was consistent across treatment temperatures.

263 Desiccation tolerance: We found a significant interaction between rainfall of origin × geography (**Fig. 4d**;
264 **Table S3**). This meant that latitudinal populations showed no association between rainfall and desiccation
265 tolerance, whereas Victorian populations from drier environments showed greater desiccation tolerance
266 (**Fig. 4d**). This result was consistent across treatment temperatures. Victorian populations from drier
267 environments therefore showed greater desiccation tolerance, suggesting local adaptation to water
268 availability.

269 To ensure that body size did not affect the association between climate of origin and each tolerance trait,
270 we conducted the same analyses, but we used population means and included wing size as a covariate.

Including wing size in the analysis did not change the strength of the association between climate of origin and tolerance traits, suggesting the patterns observed were not due mainly to variation in body size.

Hypothesis III: Heat tolerance plasticity is linked to environmental predictability

To test for an association between environmental predictability and plasticity, we focussed on heat tolerance as it showed larger variation in plasticity among populations compared to other traits (**Fig. 3**) and the most consistent association with climate (**Fig. 4**). Greater autocorrelation (i.e., predictability) of temperature across days was associated with greater plasticity for the Victorian populations, but less plasticity along latitude (**Fig. 5**). This was supported by a significant interaction between autocorrelation of mean temperature and geography (latitudinal vs Victorian populations) (**Table S4**). Victorian populations from more predictable thermal environments therefore showed greater increases in heat tolerance when they were exposed to the warmest treatment temperature (**Fig. 5**). We found the same positive trend at 13°C, but because plasticity reduced heat tolerance at the cooler treatment, populations from more predictable environments showed a smaller reduction in heat tolerance. These results suggest that Victorian populations (but not latitudinal populations) from more predictable environments showed adaptive thermal plasticity.

Discussion

Using populations of *Drosophila melanogaster*, we contrasted population variation in tolerance traits and body size across latitude along east-coast Australia with population variation at a smaller geographical scale within Victoria. Not only was among-population variation in Victoria significant for all traits, but the variation was similar in extent to that seen across latitude (*Hypothesis I*: **Figs. 2–3**). This among-population variation in tolerance traits was associated with the climate of origin, which provides evidence for local adaptation across Victorian populations (*Hypothesis II*: **Fig. 4**). Plasticity in heat tolerance was associated with temperature predictability, but only for Victorian populations (*Hypothesis III*: **Fig. 5**). Together, our results suggest that environmental heterogeneity at a relatively small geographic scale can generate local adaptation that mirrors the extent of adaptive genetic variation across 3000-km in latitude. Local-scale environmental heterogeneity can therefore generate adaptive genetic variation that increases the adaptive capacity of the metapopulation. However, we also showed that latitudinal and Victorian populations often showed contrasting patterns of adaptation, and that patterns of adaptation changed with treatment temperature (**Figs. 4–5**). Accurately predicting population responses to global change therefore needs to consider how adaptation arises across geographic scales.

Contrasting patterns of adaptation across geographic scales

While the presence of latitudinal clines suggest that extensive gene flow could transfer beneficial alleles

305 to declining populations at the broader geographical context (Meek *et al.* 2023), our results indicate that
306 this process can occur on a much smaller geographic scale. We show that beneficial alleles are likely to
307 segregate among local (and potentially fragmented) populations at a smaller scale than previous
308 anticipated, which increases the adaptive capacity of the metapopulation to global change. Given that
309 gene flow increases at smaller spatial scales (Sexton *et al.* 2024), populations could rely on locally
310 segregating adaptive genetic variation rather than the movement of adaptive alleles across broad
311 geographic scales (Meek *et al.* 2023; Sexton *et al.* 2024). However, our results raise several concerns
312 about predicting population responses to global change based on patterns of adaptation across geography.
313 First, patterns of climate adaptation often disappeared under different temperatures, which highlights the
314 need to understand adaptive potential in response to environmental variation that better represent the
315 breadth of environments created by global change. Second, associations between climate of origin and all
316 traits (except heat tolerance), as well as plasticity in heat tolerance, differed for Victoria populations
317 compared to latitudinal populations. Inferences for natural populations facing climate change are
318 therefore likely to change with geography. While heat tolerance was consistently greater in populations
319 from warmer environments, predicting population responses based on other traits and environmental
320 variables will depend on how adaptation has been shaped across geography.

321 *Segregation of adaptive genetic variation across a heterogeneous landscape*
322 Few studies have directly contrasted adaptation across geographic scales. In lodgepole pines, regional
323 variation accounted for 20% of total genetic variance in key ecological traits, suggesting that
324 environmental heterogeneity maintains genetic variation (Yeaman & Jarvis 2006). At a molecular level,
325 subtle genetic differences in a sea urchin emerged at a local scale (<100km) and were associated with sea
326 temperature, which provides evidence of local adaptive genetic differentiation (Rumberger *et al.* 2025).
327 While Hoffmann *et al.* (2001) found evidence of variation among populations for cold tolerance in *D.*
328 *melanogaster* at local scales (c.20-100km), they did not test heat tolerance or connect among population
329 variation to the environment of origin. Nadeau and Urban (2024) demonstrated variation in thermal
330 tolerance among *Daphnia* from nearby pools with different thermal conditions, but could not link this
331 variation with environmental differences, suggesting that microgeographic variation did not generate
332 patterns of adaptation. While these studies show that small-scale environmental heterogeneity can
333 generate among-population variation, they struggled to connect such variation with environmental
334 heterogeneity at the appropriate scale, or link adaptive genetic variation to key ecological traits. By
335 contrast, our study focused on a variety of climatic environments from a 300×600km area, which
336 maximised our chance of connecting patterns of local adaptation with environmental heterogeneity
337 despite the homogenising effects of gene flow that occurs regularly within 500km (Kennington *et al.*
338 2003).

Predictions about adaptation change across scales

Our key result is that our understanding of climatic adaptation changes depending on geographic scale (O'Brien *et al.* 2017). While Victorian populations mimicked broader heat tolerance trends (populations from warmer areas show higher heat tolerance), this was not the case for any other tolerance trait or body size. Therefore, our predictions are consistent for adapting to heat, but become inaccurate when considering plasticity in heat tolerance, or any other trait. This is because Victorian populations showed the opposite patterns to the latitudinal populations for cold and desiccation tolerance, and body size. Therefore, while latitudinal clines are a powerful tool for understanding adaptation to broad climatic gradients, if we scale down the same predictions, they can become wildly inaccurate and misrepresent the relationship between temperature and climatic tolerance traits. Accurately predicting the adaptive potential of natural population facing global change therefore requires understanding how adaptation occurs across geographical scales (O'Brien *et al.* 2017; Sexton *et al.* 2024; Urban *et al.* 2024).

Our results suggest that predicting adaptation to climate change based on latitudinal patterns are likely to be inaccurate at other geographical scales. Along latitude, populations from lower (warmer) latitudes showed higher heat tolerance, lower cold tolerance and smaller body size (**Fig. 4a,b,d**), suggesting that thermal tolerance and body size are selected together across latitude. While Victorian populations from warmer locations had higher heat tolerance, they contrasted with latitudinal populations by showing higher cold tolerance and larger body sizes (**Fig. 4a,d**). Selection for body size across the cline could be due to autocorrelation of environmental variables (e.g., humidity) or genetic factors (e.g., inversions; Rako *et al.* 2006) driving correlated trait responses to latitude. Compared to latitudinal populations, Victorian populations experienced lower humidity and greater temperature fluctuations (**Fig. 1b-c**), which could increase heat tolerance while creating different changes in body size and cold tolerance (Hangartner *et al.* 2020). In support of this, Victorian populations showed greater adaptive genetic variation in desiccation tolerance than latitudinal populations (**Fig. 2d, 4d**). Selection could therefore favour different combinations of traits away from the coast compared to across latitude. Accurately predicting species' responses to climate change then requires understanding how populations from across a species' range, rather than from a subset of environments, respond to environmental variation that includes climate extremes (Matesanz & Ramírez-Valiente 2019; Walter *et al.* 2020; Meek *et al.* 2023; Rumberger *et al.* 2025). Our results also suggest that understanding the drivers of body size, particularly latitudinal trends (i.e., Bergmann's rule), requires further consideration of correlated responses in other traits (e.g., desiccation tolerance) and environmental factors other than temperature.

Temperature-dependent patterns of adaptation

Compared to latitudinal populations, patterns of adaptation changed across treatment temperatures to a greater extent for Victorian populations (**Fig. 4**). The cold treatment tended to reduce variation among populations, whereas the hot temperature increased variation among populations. However, greater

variation at the high temperature often weakened patterns of adaptation in desiccation and heat tolerance, and created opposing patterns to what was predicted for cold tolerance (populations from warmer environments were more cold-tolerant; **Fig. 4a**). These results suggest that across a species range, some populations are likely to be more sensitive to temperature changes than others. This is consistent with studies showing that patterns of adaptation can weaken under stressful or variable conditions (Booker 2024; Walter *et al.* 2025), which increases vulnerability to climate change (Anderson & Wadgymar 2020). Accurately predicting the response of natural populations to global change therefore requires considering how locally adapted populations will vary in their response to the particular conditions created by environmental change.

Selection for plasticity across Victoria but not across latitude

Victorian populations from locations where temperature was more predictable across days showed greater thermal plasticity (**Fig. 5**). These results support theory and empirical observations that environmental predictability generates adaptive plasticity (Reed *et al.* 2010; Diaz *et al.* 2020; Leung *et al.* 2020; Gallegos *et al.* 2024), which helps populations adapt to thermal variation. Local adaptation across a heterogeneous landscape can therefore generate variation in plasticity, which could determine population responses to environmental change (Vinton *et al.* 2022). While such adaptive plasticity can help populations to buffer familiar thermal variation, it could also increase their vulnerability to unpredictable changes in temperature created by global change (Ashander *et al.* 2016). Furthermore, latitudinal populations showed no association between thermal predictability and plasticity, suggesting that not all predictable environments will favour the evolution of adaptive plasticity. Understanding how adaptive plasticity evolves across a heterogeneous landscape, and then determining the consequences for persisting under environmental change remains a substantial challenge for predicting population responses to global change (Meek *et al.* 2023).

Conclusions

We show that adaptive genetic variation that emerges at a relatively small-scale is comparable to that found across a broad latitudinal gradient. Such local adaptive genetic variation improves the adaptive potential of species responding to rapid environmental change, but changes in environment, such as exposure to temperature extremes, can obscure the detection of such adaptive genetic variation. More broadly, our results support the idea that local adaptation at smaller geographic scales can generate adaptive genetic variation, which means that adaptive alleles can be harnessed more efficiently than previously anticipated. Decisions on managing landscapes should then aim to maintain the processes that generate adaptative variation at multiple geographic scales. By focusing on conserving environmental heterogeneity, including at local scales, adaptive potential to environmental change can be enhanced.

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Tables & Figures

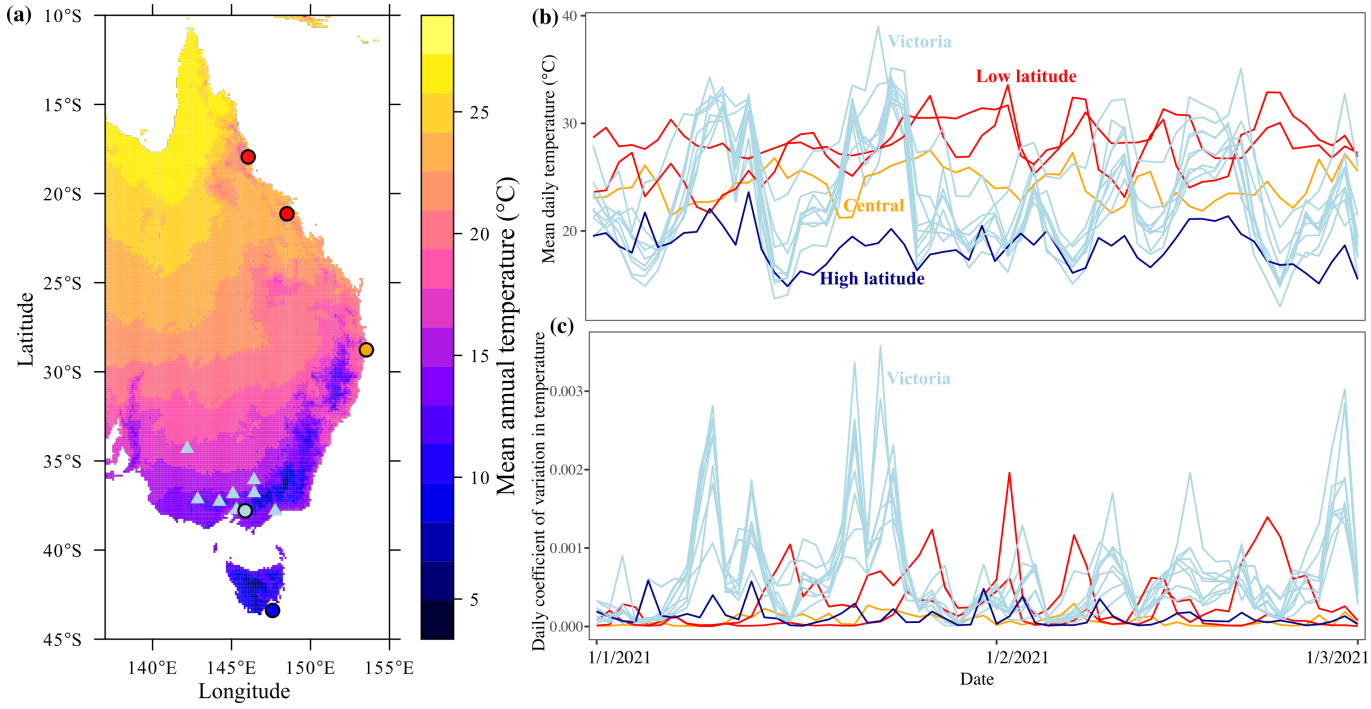


Fig. 1 (a) Map of sampling locations along east coast Australia (circles; n=5) compared to locations within Victoria (triangles; n=8). Shading represents the average mean annual temperature, where lighter shading (warmer colours) depict warmer areas. **(b)** Mean daily temperature for the sampled locations. **(c)** Daily coefficient of variation in temperature for all sampled locations. Data produced using *NicheMapR* (Kearney & Porter 2020).

Table 1 ANOVA summaries for latitudinal and Victorian populations for the four traits (**a-d**). Parameters in bold are significant at p<0.05. Temp = treatment temperature, and Pop = population.

			(a) Cold		**(b)** Heat		**(c)** Desiccation		**(d)** Wing size	
Geog-raphy	**Parameter**	***df***	χ^2	**P**	χ^2	**P**	χ^2	**P**	χ^2	**P**
Latitude	Intercept	1	582.7	**<0.001**	1531.6	**<0.001**	7600.0	**<0.001**	627800.2	**<0.001**
	Temp.	2	498.1	**<0.001**	1004.9	**<0.001**	13.9	**<0.001**	2777.4	**<0.001**
	Pop.	4	33.4	**<0.001**	69.2	**<0.001**	12.4	**0.014**	482.6	**<0.001**
	Temp×Pop	8	10.5	0.234	30.4	**<0.001**	6.8	0.558	39.6	**<0.001**
Victoria	Intercept	1	868.9	**<0.001**	1723.5	**<0.001**	10869.8	**<0.001**	940758.3	**<0.001**
	Temp.	2	589.5	**<0.001**	1789.7	**<0.001**	26.1	**<0.001**	4142.1	**<0.001**
	Pop.	7	24.0	**0.001**	73.4	**<0.001**	42.6	**<0.001**	28.0	**<0.001**
	Temp×Pop	14	32.0	**0.004**	45.8	**<0.001**	13.0	0.527	20.1	0.126

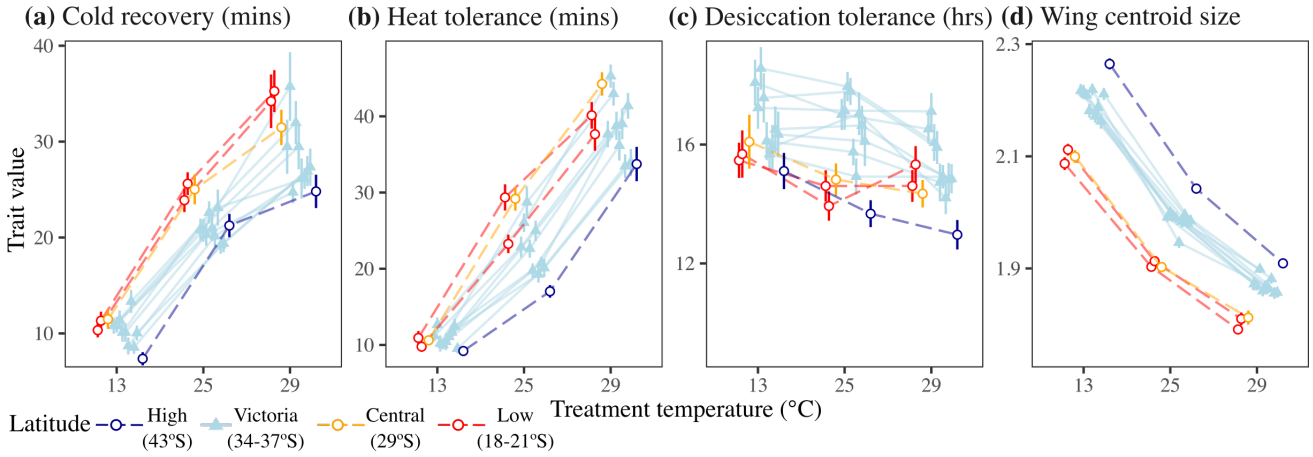
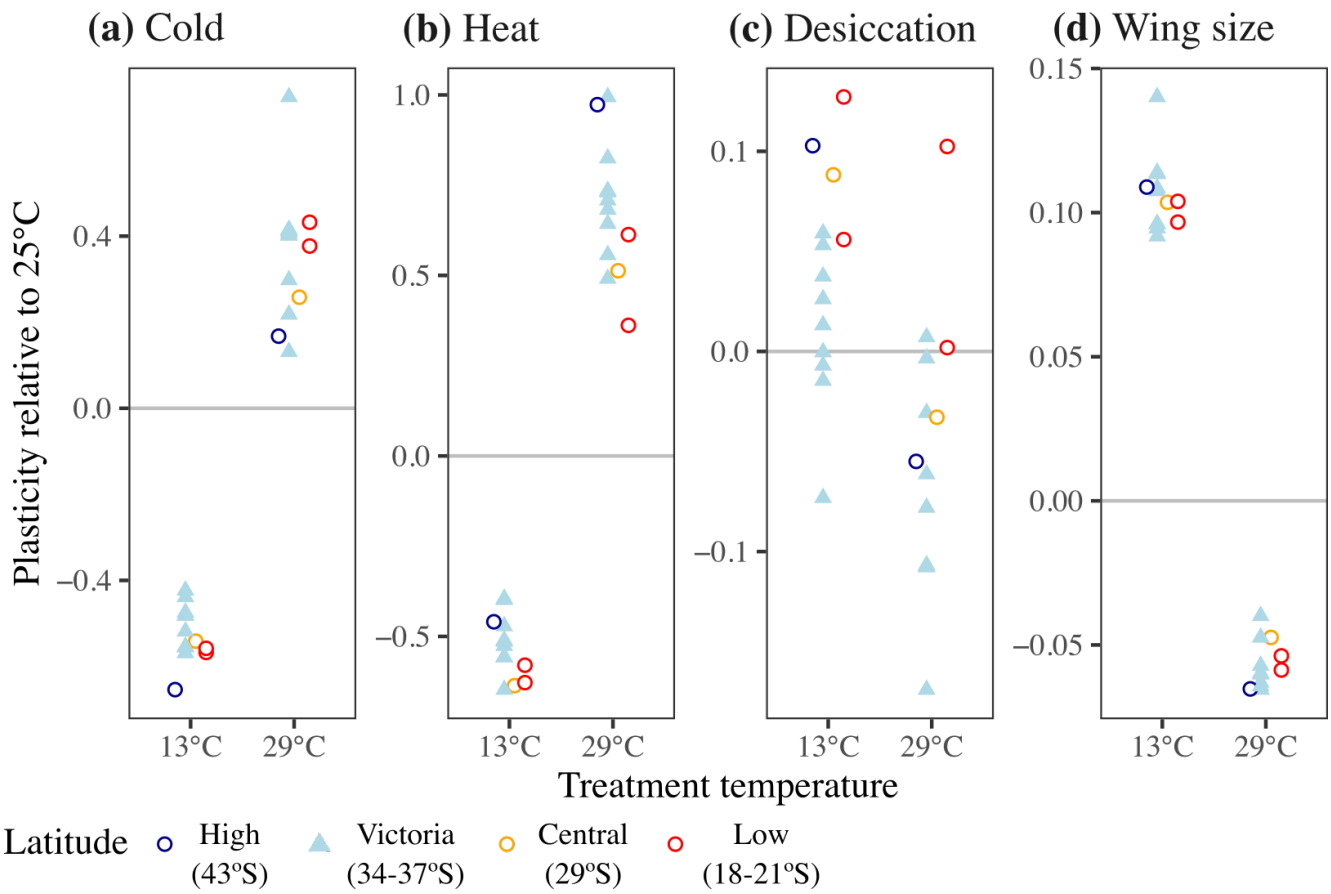


Fig. 2 The effect of treatment temperature on stress tolerance and body size for populations along latitude (open circles) and within Victoria (closed circles). We measured **(a)** cold tolerance as chill coma recovery time (mins), **(b)** heat tolerance as heat knockdown time (mins), **(c)** desiccation tolerance (hours), and **(d)** body size as wing centroid size. Points and error bars represent the marginal means (±1SE). Data points are offset for clarity.



682

683 **Fig. 3** Population variation in thermal plasticity for each trait (**a-d**). Triangles represent Victorian
684 populations, and circles the latitudinal populations. Horizontal grey lines represent the control conditions
685 (25°C), and the points represent the plasticity for each population as the change in phenotype from the
686 control condition. Data points are offset for clarity.

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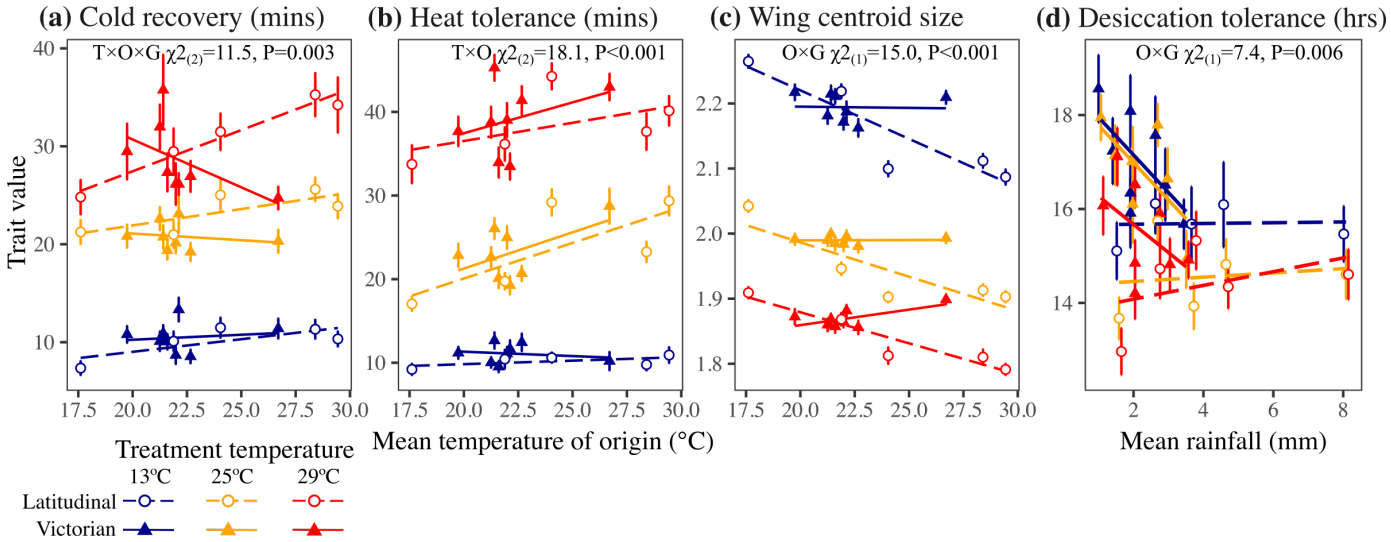

Fig. 4 Association between climate of origin and each trait (**a–d**) after exposure to three temperatures (different colours). Panels **a–c** compare mean daily temperature to cold tolerance, heat tolerance and wing size, while panel **d** compares average daily rainfall to desiccation tolerance. Points and error bars represent the marginal means (±1SE). Significant interactions are presented at the top of each panel, where T=treatment temperature, O=environment of origin and G=geographical comparison (latitude vs Victoria). **Table S3** contains the full ANOVA summaries. **Fig. S3** separates out the treatment temperatures for desiccation tolerance to observe the patterns more easily.

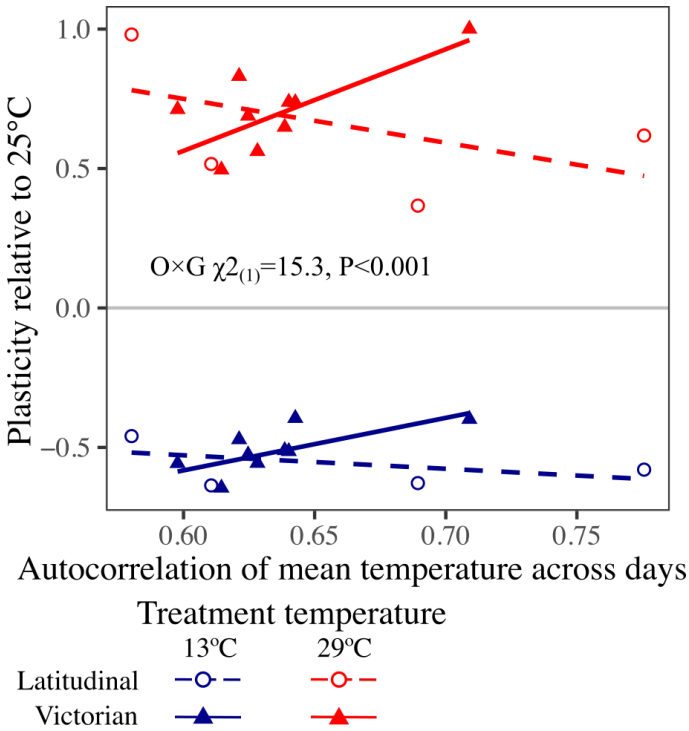

Fig. 5 Association between autocorrelation of mean temperature across days (for the sampled locations), with plasticity in heat tolerance. Plasticity is calculated as heat tolerance relative to the 25°C control temperature. Triangles represent Victorian populations, and open circles the latitudinal populations. The significant O×G interaction is presented, where O=environment of origin and G=geographical comparison (latitude vs Victoria). This provides evidence that Victorian and latitudinal populations differ in their relationship between autocorrelation in temperature and thermal plasticity. **Table S4** contains the full ANOVA summary.