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

Greg M. Walter[1,2], Avishikta Chakraborty[1,3], Fiona E. Cockerell[1], Vanessa Kellermann[1,4], Lesley A. Alton[1], Craig R. White[1], Matthew D. Hall[1], Carla M. Sgrò[1]*

[1] *School of Biological Sciences, Monash University, Melbourne 3800.*
[2] *School of Natural Sciences, University of Tasmania, Hobart 7001.*
[3] *Department of Genetics, Evolution & Environment, University College London, London WC1E 6BT.*
[4] *Department of Environment and Genetics, La Trobe University, Melbourne 3086.*

* Corresponding Author: Greg M. Walter
Email: greg.walter@utas.edu.au
Address: Life Sciences Building, University of Tasmania,
Churchill Ave, Sandy Bay, Hobart 7005 Australia

Abstract

While climate adaptation is typically quantified across broad environmental gradients, adaptation at small geographic scales is not typically considered. If local-scale environmental heterogeneity has a similar capacity to generate patterns of adaptation to that found across broad gradients, then we currently underestimate adaptive capacity to global change. We quantified population variation in climate tolerance traits and their plasticity for five populations of *Drosophila melanogaster* from a 3000-km latitudinal gradient, which we contrasted with eight local populations from an environmentally heterogeneous 600×300km area. Population variation in stress tolerance at the local scale was comparable to that across latitude. Consistent with local adaptation, populations from warmer and drier environments showed greater heat and desiccation tolerance, and populations from more predictable environments showed greater plasticity. Adaptation at smaller geographic scales can therefore contribute more to adaptive capacity than originally thought. However, patterns of adaptation often changed across geography, 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 alleles 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 adaptation, 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). Studies of climatic adaptation typically test for latitudinal clines in stress tolerance traits that reflect adaptation to broad climatic gradients (Huey *et al.* 2000; Hoffmann *et al.* 2002; Etterson 2004; Trotta *et al.* 2006; Sgrò *et al.* 2010; Lasne *et al.* 2018; Ren *et al.* 2020; Lush *et al.* 2024). However, we critically lack tests of whether these same patterns of adaptation emerge at smaller geographic scales (e.g., tens or hundreds of kilometres), where gene flow

is more likely to move adaptive alleles among populations and increase the adaptive capacity of populations (Stotz *et al.* 2021; Alvarado *et al.* 2022). While the idea that environmental heterogeneity can generate adaptation at different geographic scales has been around for some time (e.g., Cowling & Pressey 2001; Pressey *et al.* 2007; Sgrò *et al.* 2011), the extent of adaptation across geographic scales is rarely tested directly. Studies have shown subtle associations between temperature and local genetic differentiation despite little population structure across latitude (Rumberger *et al.* 2025), and that local adaptation can disrupt latitudinal patterns (Hice *et al.* 2012). However, we lack direct contrasts of climate adaptation across geographic scales, which limits our ability to predict the adaptive capacity of populations facing global change.

The interplay between dispersal (i.e., gene flow) and selection across geography determines the extent of adaptive genetic differentiation at all geographical scales (Slatkin 1987; García-Ramos & Kirkpatrick 1997; Garant *et al.* 2007; DeMarche *et al.* 2019; Booker 2024). Across geography there are two key spatial scales based on the relative strength of gene flow versus selection. At a broad (latitudinal) scale**,** distances are greater than typical migration events and even relatively weak differences in selection can create population differentiation (Wright 1943; DeMarche *et al.* 2019). By contrast, at local scales, defined as distances where substantial gene flow occurs (within hundreds of km for highly dispersive species, such as *Drosophila melanogaster*; Gockel *et al.* 2001), local adaptation can only arise when strong differences in selection overcome the homogenizing effects of gene flow (Slatkin 1987; García-Ramos & Kirkpatrick 1997; Lenormand 2002; Richardson *et al.* 2014). Adaptation is also determined by the nature of selection, which can fundamentally differ across geographic scales.

Distinct selective pressures can be generated at different spatial scales. Adaptation along latitude arises in response to broad abiotic gradients, particularly changes in temperature and seasonality that are autocorrelated and create consistent directional selection across large distances (Endler 1977; Sunday *et al.* 2011). By contrast, environmental heterogeneity at a local scale can be created by microhabitat variation, topographic complexity, or localized climatic conditions that create spatial mosaics of selection within the gene flow neighbourhood (Garant *et al.* 2007; Richardson *et al.* 2014). Disruptive selection or changes in directional selection at local scales can then make it difficult to predict patterns of adaptation at local versus broad scales (Lenormand 2002). Consequently, adapting to different combinations of selective pressures across geography can create trade-offs among traits that are likely to determine how adapting to one environmental variable (e.g., heat) determines the extent to which adaptation to other variables (e.g., moisture stress) is possible (Agrawal 2020; Willi & Van Buskirk 2022). Given that climate change is expected to require adaptation to multiple stressors (Urban *et al.* 2024), assessing adaptation across geography needs to consider the response in different traits that may interact with each other.

85 In addition to adaptation, plastic changes in climate tolerance traits also help populations buffer global
86 change (Reed *et al.* 2011; Gunderson & Stillman 2015; Magozzi & Calosi 2015). Environments that
87 are variable and predictable favour the evolution of greater plasticity (Scheiner 1993; Leung *et al.*
88 2020), which creates populations that differ in plasticity when they adapt to different environments
89 (Lind & Johansson 2007; Murren *et al.* 2014; Matesanz & Ramírez-Valiente 2019; Walter *et al.* 2022).
90 Lower plasticity but greater overall climate tolerance is then expected to evolve in environments that
91 are either consistently harsh, or variable but unpredictable (Reed *et al.* 2010; Botero *et al.* 2015). Both
92 adaptation and plasticity can therefore vary across spatial scales (and interact with each other) to
93 determine population responses to environmental change. Local-scale environmental heterogeneity can
94 favour plasticity that allows populations to track fine-grained variation (Baythavong 2011), while
95 broad-scale gradients may favour greater tolerance at lower (constantly warm) latitudes but greater
96 plasticity at higher latitudes where temperatures are more variable (Janzen 1967; Ghalambor *et al.*
97 2006). These scale-dependent patterns in the balance between adaptation and plasticity remain largely
98 untested but are critical for predicting population responses to global change.

99 To contrast the extent of adaptation in climate tolerance traits across geographic scales, we focus on
100 geographic variation in populations of the vinegar fly, *Drosophila melanogaster*. Since its introduction
101 to the east coast of Australia, c.100 years ago, *D. melanogaster* has developed latitudinal clines in
102 numerous traits, including body size and thermal tolerance (reviewed in Hoffmann & Weeks 2007;
103 Lasne *et al.* 2018). Molecular data suggest that population structure is weak along latitude due to some
104 level of gene flow at distances up to 1500km (Gockel *et al.* 2001; Kennington *et al.* 2003). Such broad
105 patterns of adaptation and high potential for gene flow mean that *D. melanogaster* provides a powerful
106 conservative test of whether small-scale geographic variation can generate and maintain local
107 adaptation. This is because local adaptation should only emerge if differences in selection at local
108 scales can outweigh the high connectivity present among populations of *D. melanogaster*. We sampled
109 five populations of *D. melanogaster* across 3000-km in latitude along east coast Australia (18°S–
110 43°S), which we compared to eight populations sampled from a variety of climates within a
111 600km×300km area in Victoria (**Fig. 1a**). Compared to the latitudinal populations, locations sampled
112 in Victoria showed greater fluctuations in temperature, both across days (**Fig. 1b**) and within days
113 (**Fig. 1c**). Using a laboratory manipulative experiment, we tested how populations across geography
114 varied in body size and climate tolerance traits, as well as in their plasticity to temperature treatments.

115 We test three hypotheses to contrast the extent of adaptive differentiation in climate tolerance across
116 geography: (I) We would detect variation among populations in Victoria for climate tolerance traits
117 and their plasticity, but variation across latitude would be greater. (II) If the among-population
118 variation is adaptive, associations between climate of origin and tolerance traits would be similar for
119 latitudinal and Victorian populations. (III) Population variation in thermal plasticity would be

120 associated with environmental variation and predictability. If local environmental heterogeneity
121 generates adaptive differentiation that approaches what is observed across latitude, then we could be
122 underestimating the adaptive capacity of local populations facing global change. Adaptation across
123 latitude should generate higher heat tolerance, lower cold and desiccation tolerance, and smaller body
124 sizes at lower latitudes. More constant higher temperatures at low latitudes should also generate lower
125 plasticity (Walter *et al.* 2025). If local environmental heterogeneity generates adaptation within
126 Victoria, variation among populations should mimic latitudinal patterns: populations from warmer,
127 less variable environments would converge on the trait values of lower-latitude populations. However,
128 given higher temperature fluctuations in Victoria, environments that are warmer, and more variable
129 could generate greater plasticity that contrasts to latitudinal trends.

130

131

132 **Methods**

133 *Collection and maintenance of experimental populations*
134 To ensure our estimates of among-population variation would be conservative, we sampled flies in
135 Autumn, which is after the summer peak population size that has the greatest potential for
136 homogenization of populations via dispersal (Shpak *et al.* 2010; Behrman *et al.* 2015). In March–May
137 2021, we collected 20–50 field-inseminated female *D. melanogaster* from thirteen locations in
138 Australia using sweep nets over fruit waste. For the five latitudinal populations, we sampled flies at
139 fruit farms in Tasmania, New South Wales, Victoria and Northern Queensland (**Fig. 1a**; **Table S1**). In
140 Victoria, we sampled flies from discarded grape skins at eight wineries from a variety of climates (**Fig.
141 1a**; **Table S1**). We chose wineries due to the abundance of breeding habitat at the same time of year,
142 allowing us to sample broadly across the state within a short period. For each population, we generated
143 a mass-bred population in the lab from 19-60 isofemale lines (mean=44.6; **Table S1**), which we
144 maintained at large sizes (1000-1500 individuals) in five 300mL bottles. We kept all populations at
145 25°C for 12 generations (c.9 months) to: (1) remove transgenerational environmental effects to focus
146 on genetic differences among populations, (2) ensure that genotypes were sufficiently mixed by
147 breaking up any linkage disequilibrium present in the original population (Kellermann *et al.* 2015),
148 and (3) avoid conducting experiments during COVID-19 lockdowns. Details of population
149 establishment and maintenance are in **Methods S1**.

150 *Manipulative experiment*
151 In January 2022, we conducted a laboratory experiment by rearing larvae from all 13 populations
152 under three temperature treatments (13, 25 and 29°C), and measuring wing size and tolerance traits
153 (heat, cold and desiccation) on emergent adults. 25°C represents control conditions, and 13 and 29°C

154 represent the cold and hot margins that larvae in natural populations are often exposed to in winter and
155 summer, respectively. For each population, we placed c.200 adult flies in each of two laying pots with
156 a c.3mm layer of food and let them lay for 6 hours. We then transferred 20 eggs into each vial (n=36–
157 42 vials/population/treatment; **Table S2**) containing 7mL of food and left larvae to develop in their
158 treatment. To keep replication feasible, we split the experiment into two temporal blocks so that adults
159 emerged 21–28 January (block 1) and 1–8 March (block 2). For each block, we conducted 2–3 rounds
160 of egg-picks for each treatment over 4–5 days to maximise the overlap in eclosion across treatments.
161 We timed the egg picks according to pilot experiments so that adult flies would emerge
162 simultaneously. We used 1456 vials in total (n=455–546 vials/treatment).

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

167 *Stress tolerance assays and body size measurements*

168 We measured females, which we collected under light CO_2 anaesthesia, let them recover (48 hours)
169 and then used established methods to measure heat, cold, or desiccation tolerance, or body size
170 (Hoffmann *et al.* 2002; Lasne *et al.* 2018). We measured 30 females per population in each assay,
171 which we split across the two blocks. Different individuals were measured in each assay. To assay heat
172 tolerance, we measured heat knockdown by putting individual females into sealed 5mL glass vials,
173 submerging them in a 39°C water bath and measuring the time for each female to cease moving. To
174 assay cold tolerance, we measured chill-coma recovery by placing individual females into sealed
175 1.5mL plastic tubes that we submerged in a 0°C water bath for 4 hours and measured the time they
176 took to regain movement. To measure desiccation tolerance, we put individual females in unsealed
177 5mL vials placed in sealed glass tanks containing silica gel (to maintain relative humidity <5%) and
178 recorded the time they took to cease moving. To measure wing size, we removed the right wing of 30
179 females, mounted them on a microscope slide and photographed them (M60 stereo microscope, Leica,
180 Heerbrugg, Switzerland). We used *imageJ* to quantify 10 vein intersection landmarks (**Fig. S1**) and
181 estimate centroid size.

182 *Statistical analyses*

183 We used *R* (v.4.3.2) statistical software for all analyses (R Core Team 2024). We applied linear mixed
184 models using *glmmTMB* (Brooks *et al.* 2017) and checked model assumptions using *DHARMa* (Hartig
185 2022). We tested for significant interactions using type-III ANOVA.

186 *Hypothesis I: Testing for among-population variation in tolerance traits and body size*
187 To test whether variation among populations was significantly different across geography, we applied
188 the linear mixed model

189 $$y_{ijkl} = G_i + p_{j(i)} + r_k + e_{l(ijk)} \,, \qquad (1)$$

190 where geography (G_i; latitude vs Victoria) is a fixed effect. Replicate run nested within block was a
191 random effect (r_k) and $e_{l(ijk)}$ is the residual. We applied equation 1 to each trait and treatment
192 temperature separately. We included population ($p_{j(i)}$) as a random effect and used log-likelihood ratio
193 to test the null model that estimates a single variance among populations and so assumes equal
194 variance across regions, i.e., $p_{j(i)} \sim N(0, \sigma^2)$, with the alternative model estimating an among-
195 population variance for each geographical region, and so $p_{j(i)} \sim N(0, \sigma^2_{G[i]})$ where *i* indexes region.
196 Significant differences between models suggest that among-population variance differs between
197 latitude vs Victoria. While we analysed each temperature treatment separately, including temperature
198 in the model produced the same results.

199 Next, we tested whether variation among populations was significant within each region, and whether
200 populations (within region) varied significantly in their response to temperature treatments. We used
201 equation 1, but analysed latitudinal and Victorian populations separately, included population and
202 temperature as fixed effects and tested for significant population×temperature interactions. Replicate
203 run was the only random effect.

204 *Hypotheses II: Testing whether among-population variation is adaptive*
205 For all 13 locations, we extracted fine-scale rainfall (daily) and temperature (hourly) data using
206 *Nichemapr* (Kearney & Porter 2020) for the three summer months preceding sampling. To quantify
207 temperature variation, we calculated standard deviation across the three months. To quantify
208 temperature predictability, we used the *acf* function to calculate autocorrelation of daily mean
209 temperature across the three months. To test for associations between climate of origin and assayed
210 traits, we used

211 $$y_{ijklm} = O \times G_i \times T_j + p_{k(ij)} + r_l + e_{m(ijkl)} \,, \qquad (2)$$

212 where the three-way interaction of the fixed effects ($O \times G_i \times T_j$) includes environment of origin (O)
213 measured at the population locations (continuous variable), the geographical comparison (G_i; Victoria
214 vs latitude) and treatment temperature (T_j). Random effects included the *k*th population nested within
215 treatment temperature and geography ($p_{k(ij)}$) to avoid pseudoreplication, and replicate block (r_l).
216 $e_{m(ijkl)}$ is the residual. We applied equation 2 to each trait separately. We included mean temperature

as the environmental covariate (O) for analysing thermal tolerance and body size, and mean daily rainfall for 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 the trait and environment of origin.

Hypothesis III: Testing whether environment determines plasticity

To compare differences in plasticity, we extracted the population marginal means from the second application of equation 1 using *emmeans* (Lenth 2019), and then calculated plasticity using: $plasticity = \frac{(treatment\ mean - control\ mean)}{control\ mean}$, which represents plasticity as the proportional change in phenotype from the 25°C control treatment. This removes population differences in the trait to focus on estimating plasticity of each population (Valladares *et al.* 2006). We then used equation 2 with environmental variation and predictability traits as the covariate, and plasticity as the response variable.

Results

Hypothesis I: High variation among Victorian populations for tolerance traits and plasticity

Only body size showed greater variation across latitude compared to among Victorian populations (**Fig. 2; Table 1a**). For all tolerance traits, variation among populations was not significantly different for latitudinal versus Victorian populations (**Fig. 2**; **Table 1a**). The magnitude of differences among Victorian populations was therefore similar to that observed across latitude for heat and cold tolerance, and consistently larger than latitudinal populations for desiccation resistance. Latitudinal populations showed >3-fold greater differences than Victorian populations for wing size (**Table 1b**).

Temperature created significant differences in each trait. Flies exposed to colder temperatures were larger, more cold tolerant and less heat tolerant (**Fig. 2**). Within each geographic scale, we found significant variation among populations in all tolerance traits and body size (**Table 1c**). Statistically significant interactions between population and temperature quantifies how populations vary in response to treatments, and therefore reflects population variation in plasticity. Victorian populations only showed significant temperature×population interactions for cold and heat tolerance, whereas latitudinal populations showed significant interactions for heat tolerance and wing size (**Table 1c**). Desiccation tolerance showed no significant interaction. Both Victorian and latitudinal populations

249 therefore displayed significant variation in thermal plasticity that were consistent across hot and cold
250 treatments (**Fig. 3**).

251 *Differences in climate across geography*
252 While temperatures were higher and more consistent at lower latitudes, Victorian locations
253 experienced greater variation in temperature across days (**Fig. 1b**) and within each day (**Fig. 1c**).
254 Rainfall was lower at high latitudes, ranging 193–984mm south–north, while Victorian locations
255 varied between 130–428mm (**Table S1**). Correlations between mean temperature, rainfall and
256 temperature predictability were stronger across latitude (0.81, 0.84) than Victoria (-0.06, -0.37),
257 suggesting that climate was more strongly autocorrelated across latitude (**Fig. S3**).

258 *Hypothesis II: Population variation was associated with climate of origin*
259 To test whether among-population variation in tolerance traits and body size was adaptive, we
260 quantified associations between climate of origin with the traits measured in the laboratory. Below we
261 summarise the results for each trait.

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

269 Heat tolerance: We found no significant effect of geography, suggesting that associations between
270 temperature and heat tolerance were consistent for Victorian and latitudinal populations (**Fig. 4b;**
271 **Table S3**). While there was no strong association between temperature of origin and heat tolerance at
272 13°C, at the warmer treatments, populations from warmer locations showed higher heat tolerance **(Fig.**
273 **4b)**, creating a positive association supported by a significant between temperature of origin and
274 temperature treatment (**Table S3**). We therefore found evidence of local adaptation in warmer
275 treatments, which was consistent for Victorian and latitudinal populations.

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

282 Desiccation tolerance: Latitudinal populations showed weak associations between rainfall and
283 desiccation tolerance, whereas Victorian populations from drier environments showed greater
284 desiccation tolerance that was consistent across treatment temperatures (**Fig. 4d**). This was supported
285 by a significant rainfall of origin×geography interaction (**Table S3**). Victorian populations from drier
286 environments therefore showed greater desiccation tolerance, suggesting local adaptation to water
287 availability.

288 To ensure that body size did not affect the association between climate of origin and each trait, we
289 conducted the same analyses, but used population means and included wing size as a covariate. This
290 did not change the strength of the association between climate of origin and tolerance traits, suggesting
291 the patterns observed were not due mainly to variation in body size.

292 *Hypothesis III: Heat tolerance plasticity is linked to environmental predictability*
293 To test for an association between environmental variation and plasticity, we focussed on heat
294 tolerance as it showed larger variation in plasticity among populations compared to other traits (**Fig. 3**)
295 and the most consistent association with climate (**Fig. 4**). In the hot treatment, greater predictability of
296 temperature across days was associated with larger increases in heat tolerance for the Victorian
297 populations, but smaller increases in heat tolerance along latitude (**Fig. 5a**). This was supported by a
298 significant interaction between autocorrelation of mean temperature and geography (**Table S4**). In the
299 cold treatment, we found the same pattern, but because heat tolerance was overall lower (**Fig. 2b**),
300 greater predictability was associated with a smaller decrease in heat tolerance for Victorian
301 populations, but a larger decrease for latitudinal populations (**Fig. 5a**). We found no association
302 between the amount of temperature variation and plasticity (**Fig. 5b**). Victorian populations from more
303 predictable thermal environments therefore showed more adaptive plasticity (greater plasticity in the
304 hot treatment, less plasticity in the cold treatment; **Fig. 5**). By contrast, latitudinal populations showed
305 weaker, opposing trends.

306

307

308 **Discussion**

309 Using populations of *Drosophila melanogaster*, we contrasted population variation in tolerance traits
310 and body size across latitude along east-coast Australia with population variation at a smaller
311 geographical scale within Victoria. Not only was among-population variation in Victoria significant
312 for all traits, but the variation was similar in extent to that seen across latitude (*Hypothesis I*: **Figs. 2–**
313 **3**). This among-population variation in tolerance traits was associated with the climate of origin, which
314 provides evidence for local adaptation across Victorian populations (*Hypothesis II*: **Fig. 4**). Population
315 variation in heat tolerance plasticity was more strongly associated with temperature predictability

316 across Victoria than across latitude, which showed weaker opposing trends (*Hypothesis III*: **Fig. 5a**).
317 Together, our results suggest that environmental heterogeneity at a relatively small geographic scale
318 can generate local adaptation that mirrors the extent of latitudinal adaptation. Local-scale
319 environmental heterogeneity can therefore generate adaptive differentiation that can increase the
320 adaptive capacity of the metapopulation. However, latitudinal and Victorian populations often showed
321 contrasting patterns of adaptation (**Figs. 4–5**), suggesting that predicting population responses to
322 global change needs to consider how adaptation arises across geography.

323 *Contrasting patterns of adaptation across geographic scales*

324 We show that adaptive divergence among populations can occur at a smaller scale than previously
325 anticipated, which increases the adaptive capacity of the metapopulation to global change. Given that
326 gene flow increases at smaller spatial scales (Sexton *et al.* 2024), populations could rely on locally
327 segregating adaptive alleles rather than their movement across broad geographic scales (Meek *et al.*
328 2023; Sexton *et al.* 2024). However, our results raise several concerns about predicting population
329 responses to global change based on patterns of adaptation across geography. First, patterns of climate
330 adaptation often disappeared under different temperatures, which highlights the need to understand
331 adaptive potential in response to environments that better represent global change scenarios. Second,
332 associations between climate of origin and all traits (except heat tolerance), as well as plasticity in heat
333 tolerance, differed for Victoria populations compared to latitudinal populations. Inferences for natural
334 populations facing global change are therefore likely to change with geography.

335 *Adaptive differentiation across a heterogeneous landscape*

336 Few studies have directly contrasted adaptation across geographic scales. In lodgepole pines, regional
337 variation accounted for 20% of total genetic variance in key ecological traits, suggesting that
338 environmental heterogeneity maintains genetic variation (Yeaman & Jarvis 2006). At a molecular
339 level, subtle adaptive genetic differences in a sea urchin at a local scale (<100km) were associated with
340 temperature despite little population structure across latitude (Rumberger *et al.* 2025). While
341 Hoffmann *et al.* (2001) found evidence of variation among populations for cold tolerance in *D.*
342 *melanogaster* at local scales (c.20-100km), they did not test heat tolerance or connect among
343 population variation to the environment of origin. Nadeau and Urban (2024) demonstrated variation in
344 thermal tolerance among *Daphnia* from nearby pools, but this variation was not associated with
345 environmental differences, suggesting that microgeographic variation did not generate patterns of
346 adaptation. While these studies suggest among-population variation can be generated at local scales,
347 they either struggled to connect such variation with environmental heterogeneity, or link adaptive
348 genetic variation to key ecological traits. By contrasting populations across latitude with local
349 populations from variety of climates, we showed how environmental heterogeneity generates adaptive
350 trait divergence at different geographical scales.

Predictions about adaptation change across geography

Our key result is that understanding climatic adaptation changes with environments across geography. While Victorian populations mimicked broader heat tolerance trends (populations from warmer areas showed higher heat tolerance), this was not the case for any other tolerance trait or body size. Populations from lower (warmer) latitudes showed higher heat tolerance, lower cold tolerance and smaller body size (**Fig. 4a-c**), suggesting that thermal tolerance and body size are selected together across latitude. Correlated trait responses to latitude could be due to stronger autocorrelation of environmental variables (**Fig. S3**) and/or genetic factors (e.g., inversions; Rako *et al.* 2006) across latitude, whereas a different combination of traits could be favoured away from the coast. While Victorian populations from warmer locations showed higher heat tolerance (consistent with latitudinal trends), they contrasted with latitudinal trends by showing higher cold tolerance and larger body sizes (**Fig. 4**), and stronger associations between moisture availability and desiccation resistance (**Fig. 4d**). Although latitudinal clines are a powerful tool for understanding adaptation to broad climatic gradients, they likely represent adaptation to a specific combination of environmental variables. Broad patterns of adaptation are then likely to provide inaccurate predictions for population responses to global change when applied to different geographic scales or environments (O'Brien *et al.* 2017; Cornwell *et al.* 2021; Sexton *et al.* 2024; Urban *et al.* 2024).

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**). Across a species range, some populations could therefore be more sensitive to temperature changes than others (Walter *et al.* 2020). 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.

Adaptive differentiation in plasticity across Victoria but not across latitude

While variation or predictability in temperature was weakly associated with plasticity for latitudinal populations, Victorian populations from locations where temperature was more predictable across days showed greater thermal plasticity (**Fig. 5**). Given that Victorian populations experience greater variation in temperature than latitudinal populations, these results support theory and empirical

386 observations that variable and predictable environments favour adaptive plasticity (Reed *et al.* 2010;
387 Diaz *et al.* 2020; Leung *et al.* 2020; Gallegos *et al.* 2024), which helps populations adapt to thermal
388 variation. Local adaptation across a heterogeneous landscape can therefore generate variation in
389 plasticity, which could determine population responses to environmental change (Hamann *et al.* 2016;
390 Vinton *et al.* 2022). While such adaptive plasticity can help populations buffer familiar thermal
391 variation, it could also increase their vulnerability to unpredictable changes in temperature created by
392 global change (Ashander *et al.* 2016). Understanding how adaptive plasticity evolves across a
393 heterogeneous landscape, and then determining the consequences for persisting under environmental
394 change remains challenging for predicting population responses to global change (Meek *et al.* 2023).

395 *Limitations*

396 While we show that it is possible for adaptive differentiation to occur at a smaller geographic scale
397 than originally anticipated, it is important to interpret our results cautiously. Further work is needed to
398 test whether local adaptation can emerge within regions at different latitudes, or whether it is unique to
399 the greater environmental heterogeneity present at locations in Victoria. Hoffmann *et al.* (2001)
400 showed that variation among populations within regions (latitudinal extremes) was smaller than
401 latitudinal differences, suggesting that replicating populations within latitude would not change our
402 results. However, they also showed that variation within populations was greater than variation among
403 populations, and so understanding adaptive potential requires quantifying variation within versus
404 among populations across a heterogeneous landscape. Given that estimating variation among
405 populations is labour-intensive and requires replication of >5 populations, future studies should either
406 prioritise c.10 populations randomly across a heterogeneous landscape, or target fewer populations
407 from different environments and levels of heterogeneity. Disentangling how much adaptive genetic
408 variation segregates among versus within these populations would accurately quantify the adaptive
409 potential of the metapopulation to global change. Finally, our populations were maintained at large
410 sizes for c.12 generations, which means it is possible for drift or lab adaptation to influence our
411 among-population differences. However, lab adaptation and drift should weaken patterns of
412 adaptation, and so our results should represent conservative patterns of adaptation present in the field.
413 Supporting our results, a study that maintained similarly large populations in the lab found consistent
414 latitudinal clines after 20+ generations (Lasne *et al.* 2018).

415 *Conclusions*

416 We show that adaptive differentiation across a relatively small scale is comparable to that found across
417 a broad latitudinal gradient. However, patterns of adaptation changed with treatment temperature and
418 across geography, suggesting that predicting responses to global change needs to consider how
419 adaptation occurs at different geographic scales. Overall, our results support the idea that local
420 adaptation at smaller geographic scales can generate adaptive genetic differentiation that increases the

adaptive capacity of populations facing global change. Decisions on managing landscapes should then aim to conserve local-scale environmental heterogeneity to maximise the potential for local adaptation to increase the resilience of populations.

Acknowledgements

We are grateful to two anonymous reviewers whose constructive comments helped us to greatly improve this paper. We thank Ary Hoffmann for comments on a previous version. We are grateful to Carmen da Silva, Julian Beaman and Belinda van Heerwaarden for helping to sample the latitudinal populations. We thank Andrew Murch for his insights into climatic variation across Victorian wine regions. We also thank Emily Lombardi, Mia Wansbrough and Sandra Hangartner for their help with some of the assays. GMW is grateful to be supported by Australian Research Council fellowships DE200101019 and FT240100466.

References

1.
Agrawal, A.A. (2020). A scale-dependent framework for trade-offs, syndromes, and specialization in organismal biology. *Ecology*, 101, e02924.

2.
Alvarado, A.H., Bossu, C.M., Harrigan, R.J., Bay, R.A., Nelson, A.R.P., Smith, T.B. *et al.* (2022). Genotype-environment associations across spatial scales reveal the importance of putative adaptive genetic variation in divergence. *Evolutionary Applications*, 15, 1390-1407.

3.
Anderson, J.T. & Wadgymar, S.M. (2020). Climate change disrupts local adaptation and favours upslope migration. *Ecol. Lett.*, 23, 181-192.

4.
Ashander, J., Chevin, L.M. & Baskett, M.L. (2016). Predicting evolutionary rescue via evolving plasticity in stochastic environments. *Proceedings of the Royal Society B-Biological Sciences*, 283.

5.
Baythavong, B.S. (2011). Linking the spatial scale of environmental variation and the evolution of phenotypic plasticity: selection favors adaptive plasticity in fine-grained environments. *Am. Nat.*, 178, 75-87.

6.

Behrman, E.L., Watson, S.S., O'Brien, K.R., Heschel, M.S. & Schmidt, P.S. (2015). Seasonal variation in life history traits in two *Drosophila* species. *J. Evol. Biol.*, 28, 1691-1704.

7.
Bennett, S., Duarte, C.M., Marbà, N. & Wernberg, T. (2019). Integrating within-species variation in thermal physiology into climate change ecology. *Philos. Trans. R. Soc. Lond. B.*, 374, 20180550.

8.
Booker, T.R. (2024). The structure of the environment influences the patterns and genetics of local adaptation. *Evolution Letters*, 8, 787-798.

9.
Botero, C.A., Weissing, F.J., Wright, J. & Rubenstein, D.R. (2015). Evolutionary tipping points in the capacity to adapt to environmental change. *Proc. Natl. Acad. Sci. USA*, 112, 184-189.

10.
Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C., Nielsen, A. *et al.* (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal*, 9, 378-400.

11.
Byers, D.L. (2005). Evolution in heterogeneous environments and the potential of maintenance of genetic variation in traits of adaptive significance. *Genetica*, 123, 107-124.

12.
Cornwell, B., Armstrong, K., Walker, N.S., Lippert, M., Nestor, V., Golbuu, Y. *et al.* (2021). Widespread variation in heat tolerance and symbiont load are associated with growth tradeoffs in the coral *Acropora hyacinthus* in Palau. *Elife*, 10.

13.
Cowling, R.M. & Pressey, R.L. (2001). Rapid plant diversification: planning for an evolutionary future. *Proc. Natl. Acad. Sci. USA*, 98, 5452-5457.

14.
DeMarche, M.L., Doak, D.F. & Morris, W.F. (2019). Incorporating local adaptation into forecasts of species' distribution and abundance under climate change. *Global Change Biol.*, 25, 775-793.

15.
Diaz, F., Kuijper, B., Hoyle, R.B., Talamantes, N., Coleman, J.M. & Matzkin, L.M. (2020). Environmental predictability drives adaptive within- and transgenerational plasticity of heat tolerance across life stages and climatic regions. *Funct. Ecol.*, 35, 153-166.

16.
Endler, J.A. (1977). *Geographic Variation, Speciation, and Clines*. Princeton University Press, Princeton, New Jersey.

17.
Etterson, J.R. (2004). Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. I. Clinal patterns of selection along an environmental gradient in the great plains. *Evolution*, 58, 1446-1458.

18.
Gallegos, C., Chevin, L.M., Hodgins, K.A. & Monro, K. (2024). Predicting adaptation and evolution of plasticity from temporal environmental change. *Methods in Ecology and Evolution*, 16, 84-96.

19.
Garant, D., Forde, S.E. & Hendry, A.P. (2007). The multifarious effects of dispersal and gene flow on contemporary adaptation. *Funct. Ecol.*, 21, 434-443.

20.
García-Ramos, G. & Kirkpatrick, M. (1997). Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, 51, 21-28.

21.
Ghalambor, C.K., Huey, R.B., Martin, P.R., Tewksbury, J.J. & Wang, G. (2006). Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr. Comp. Biol.*, 46, 5-17.

22.
Gockel, J., Kennington, W.J., Hoffmann, A., Goldstein, D.B. & Partridge, L. (2001). Nonclinality of molecular variation implicates selection in maintaining a morphological cline of *Drosophila melanogaster*. *Genetics*, 158, 319-323.

23.
Gunderson, A.R. & Stillman, J.H. (2015). Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proceedings of the Royal Society B-Biological Sciences*, 282, 20150401.

24.
Hamann, E., Kesselring, H., Armbruster, G.F.J., Scheepens, J.F. & Stöcklin, J. (2016). Evidence of local adaptation to fine- and coarse-grained environmental variability in *Poa alpina* in the Swiss Alps. *J. Ecol.*, 104, 1627-1637.

25.
Hartig, F. (2022). DHARMa: Residual diagnostics for hierarchical (multi-Level / mixed) regression models. R package.

26.
Hice, L.A., Duffy, T.A., Munch, S.B. & Conover, D.O. (2012). Spatial scale and divergent patterns of variation in adapted traits in the ocean. *Ecol. Lett.*, 15, 568-575.

27.
Hoffmann, A.A., Anderson, A. & Hallas, R. (2002). Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecol. Lett.*, 5, 614-618.

28.
Hoffmann, A.A., Hallas, R., Sinclair, C. & Mitrovski, P. (2001). Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance, and associated traits. *Evolution*, 55, 1621-1630.

29.
Hoffmann, A.A. & Weeks, A.R. (2007). Climatic selection on genes and traits after a 100 year-old invasion: a critical look at the temperate-tropical clines in *Drosophila melanogaster* from eastern Australia. *Genetica*, 129, 133-147.

30.
Huey, R.B., Gilchrist, G.W., Carlson, M.L., Berrigan, D. & Serra, L. (2000). Rapid evolution of a geographic cline in size in an introduced fly. *Science*, 287, 308-309.

31.
Janzen, D.H. (1967). Why mountain passes are higher in the tropics. *Am. Nat.*, 101, 233-249.

32.
Kearney, M. & Porter, W.P. (2020). NicheMapR – an R package for biophysical modelling: the ectotherm and Dynamic Energy Budget models. *Ecography*, 43, 85-96.

33.
Kellermann, V., Hoffmann, A.A., Kristensen, T.N., Moghadam, N.N. & Loeschcke, V. (2015). Experimental evolution under fluctuating thermal conditions does not reproduce patterns of adaptive clinal differentiation in *Drosophila melanogaster*. *Am. Nat.*, 186, 582-593.

34.
Kennington, W.J., Gockel, J. & Partridge, L. (2003). Testing for asymmetrical gene flow in a *Drosophila melanogaster* body-size cline. *Genetics*, 165, 667-673.

35.
Lasne, C., Hangartner, S.B., Connallon, T. & Sgrò, C.M. (2018). Cross-sex genetic correlations and the evolution of sex-specific local adaptation: Insights from classical trait clines in *Drosophila melanogaster*. *Evolution*, 72, 1317-1327.

36.
Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends Ecol. Evol.*, 17, 183-189.

37.
Lenth, R.V. (2019). emmeans: Estimated Marginal Means, aka Least-Squares Means. In: *R Package* https://CRAN.R-project.org/package=emmeans.

38.
Leung, C., Rescan, M., Grulois, D. & Chevin, L.M. (2020). Reduced phenotypic plasticity evolves in less predictable environments. *Ecol. Lett.*, 23, 1664-1672.

39.
Lind, M.I. & Johansson, F. (2007). The degree of adaptive phenotypic plasticity is correlated with the spatial environmental heterogeneity experienced by island populations of *Rana temporaria*. *J. Evol. Biol.*, 20, 1288-1297.

40.
Lush, J., Sgrò, C.M. & Hall, M.D. (2024). Anticipating change: the impact of simulated seasonal heterogeneity on heat tolerances along a latitudinal cline. *Ecology*.

41.
Magozzi, S. & Calosi, P. (2015). Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Global Change Biol.*, 21, 181-194.

42.
Matesanz, S. & Ramírez-Valiente, J.A. (2019). A review and meta‐analysis of intraspecific differences in phenotypic plasticity: Implications to forecast plant responses to climate change. *Global Ecol. Biogeogr.*, 28, 1682-1694.

43.
Meek, M.H., Beever, E.A., Barbosa, S., Fitzpatrick, S.W., Fletcher, N.K., Mittan-Moreau, C.S. *et al.* (2023). Understanding local adaptation to prepare populations for climate change. *Bioscience*, 73, 36-47.

44.
Murren, C.J., Maclean, H.J., Diamond, S.E., Steiner, U.K., Heskel, M.A., Handelsman, C.A. *et al.* (2014). Evolutionary change in continuous reaction norms. *Am. Nat.*, 183, 453-467.

45.
Nadeau, C.P. & Urban, M.C. (2024). Macroecological predictors of evolutionary and plastic potential do not apply at microgeographic scales for a freshwater cladoceran under climate change. *Evolution Letters*, 8, 43-55.

46.
O'Brien, E.K., Higgie, M., Reynolds, A., Hoffmann, A.A. & Bridle, J.R. (2017). Testing for local adaptation and evolutionary potential along altitudinal gradients in rainforest *Drosophila*: beyond laboratory estimates. *Global Change Biol.*, 23, 1847-1860.

47.
Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Ann. Rev. Ecol. Evol. Syst.*, 37, 637-669.

48.
Parmesan, C. & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, 37-42.

49.
Pressey, R.L., Cabeza, M., Watts, M.E., Cowling, R.M. & Wilson, K.A. (2007). Conservation planning in a changing world. *Trends Ecol. Evol.*, 22, 583-592.

50.
R Core Team (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.

51.
Rako, L., Anderson, A.R., Sgrò, C.M., Stocker, A.J. & Hoffmann, A.A. (2006). The association between inversion *In(3R)Payne* and clinally varying traits in *Drosophila melanogaster*. *Genetica*, 128, 373-384.

52.
Razgour, O., Forester, B., Taggart, J.B., Bekaert, M., Juste, J., Ibáñez, C. *et al.* (2019). Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proc. Natl. Acad. Sci. USA*, 116, 10418-10423.

53.
Reed, T.E., Schindler, D.E. & Waples, R.S. (2011). Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conserv. Biol.*, 25, 56-63.

54.
Reed, T.E., Waples, R.S., Schindler, D.E., Hard, J.J. & Kinnison, M.T. (2010). Phenotypic plasticity and population viability: the importance of environmental predictability. *Proc. R. Soc. Lond. B.*, 277, 3391-3400.

55.
Ren, L., Guo, X., Liu, S., Yu, T., Guo, W., Wang, R. *et al.* (2020). Intraspecific variation in *Phragmites australis*: Clinal adaption of functional traits and phenotypic plasticity vary with latitude of origin. *J. Ecol.*, 108, 2531-2543.

56.
Richardson, J.L., Urban, M.C., Bolnick, D.I. & Skelly, D.K. (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends Ecol. Evol.*, 29, 165-176.

57.

Rumberger, C., Armstrong, M., Kim, M., Ponce, R., Melendez, J., DeBiasse, M. *et al.* (2025). Selection Over Small and Large Spatial Scales in the Face of High Gene Flow. *Mol. Ecol.*, 34, e17700.

58.
Scheiner, S.M. (1993). Genetics and Evolution of Phenotypic Plasticity. *Annu. Rev. Ecol. Syst.*, 24, 35-68.

59.
Sexton, J.P., Clemens, M., Bell, N., Hall, J., Fyfe, V. & Hoffmann, A.A. (2024). Patterns and effects of gene flow on adaptation across spatial scales: implications for management. *J. Evol. Biol.*, 37, 732-745.

60.
Sexton, J.P., Strauss, S.Y. & Rice, K.J. (2011). Gene flow increases fitness at the warm edge of a species' range. *Proc. Natl. Acad. Sci. USA*, 108, 11704-11709.

61.
Sgrò, C.M., Lowe, A.J. & Hoffmann, A.A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications*, 4, 326-337.

62.
Sgrò, C.M., Overgaard, J., Kristensen, T.N., Mitchell, K.A., Cockerell, F.E. & Hoffmann, A.A. (2010). A comprehensive assessment of geographic variation in heat tolerance and hardening capacity in populations of *Drosophila melanogaster* from eastern Australia. *J. Evol. Biol.*, 23, 2484-2493.

63.
Shpak, M., Wakeley, J., Garrigan, D. & Lewontin, R.C. (2010). A structured coalescent process for seasonally fluctuating populations. *Evolution*, 64, 1395-1409.

64.
Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science*, 236, 787-792.

65.
Stotz, G.C., Salgado-Luarte, C., Escobedo, V.M., Valladares, F. & Gianoli, E. (2021). Global trends in phenotypic plasticity of plants. *Ecol. Lett.*, 24, 2267-2281.

66.
Sunday, J.M., Bates, A.E. & Dulvy, N.K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proc. R. Soc. Lond. B.*, 278, 1823-1830.

67.
Trotta, V., Calboli, F.C., Ziosi, M., Guerra, D., Pezzoli, M.C., David, J.R. *et al.* (2006). Thermal plasticity in *Drosophila melanogaster*: a comparison of geographic populations. *BMC Evol. Biol.*, 6, 67.

68.
Urban, M.C., Swaegers, J., Stoks, R., Snook, R.R., Otto, S.P., Noble, D.W.A. *et al.* (2024). When and how can we predict adaptive responses to climate change? *Evolution Letters*, 8, 172-187.

69.
Valladares, F., Sanchez-Gomez, D. & Zavala, M.A. (2006). Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *J. Ecol.*, 94, 1103-1116.

70.
Vinton, A.C., Gascoigne, S.J.L., Sepil, I. & Salguero-Gómez, R. (2022). Plasticity's role in adaptive evolution depends on environmental change components. *Trends Ecol. Evol.*, 37, 1067-1078.

71.
Walter, G.M., Catara, S., Bridle, J.R. & Cristaudo, A. (2020). Population variation in early development can determine ecological resilience in response to environmental change. *New Phytol.*, 226, 1312-1324.

72.
Walter, G.M., Chakraborty, A., Cockerell, F.E., Kellermann, V., Hall, M.D., White, C.R. *et al.* (2025). Variation in temperature but not diet determines the stability of latitudinal clines in tolerance traits and their plasticity. *Proc. R. Soc. Lond. B.*, 292, 20251337.

73.
Walter, G.M., Clark, J., Cristaudo, A., Nevado, B., Catara, S., Paunov, M. *et al.* (2022). Adaptive divergence generates distinct plastic responses in two closely related *Senecio* species. *Evolution*, 76, 1229-1245.

74.
Whitlock, M.C. (2015). Modern Approaches to Local Adaptation. *Am. Nat.*, 186, S1-4.

75.
Willi, Y., Kristensen, T.N., Sgrò, C.M., Weeks, A.R., Orsted, M. & Hoffmann, A.A. (2022). Conservation genetics as a management tool: The five best-supported paradigms to assist the management of threatened species. *Proc. Natl. Acad. Sci. USA*, 119, e2105076119.

76.
Willi, Y. & Van Buskirk, J. (2022). A review on trade-offs at the warm and cold ends of geographical distributions. *Philos. Trans. R. Soc. Lond. B.*, 377, 20210022.

77.
Wright, S. (1943). Isolation by distance. *Genetics*, 28, 114-138.

78.
Yeaman, S. & Jarvis, A. (2006). Regional heterogeneity and gene flow maintain variance in a quantitative trait within populations of lodgepole pine. *Proc. R. Soc. Lond. B.*, 273, 1587-1593.

Tables & Figures

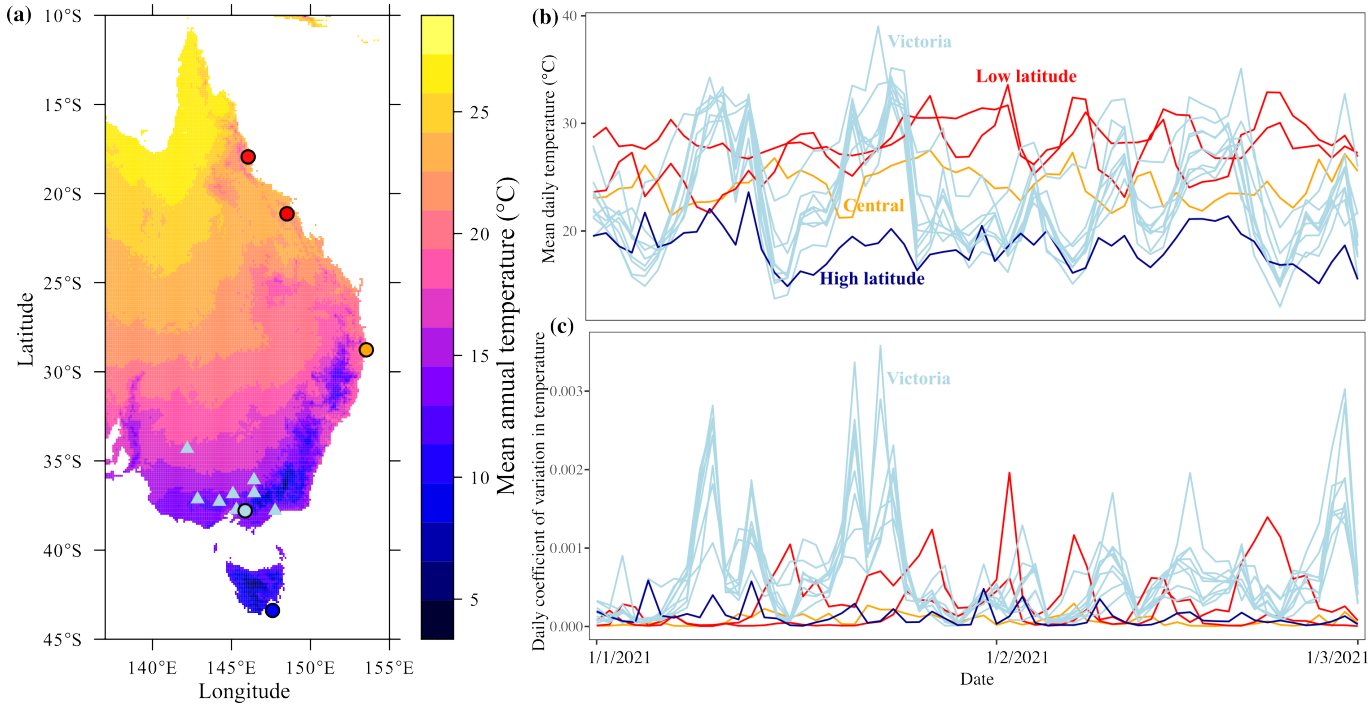

Fig. 1 (a) Map of sampling locations along east coast Australia (circles; n=5) compared to locations within Victoria (triangles; n=8). Shading represents the average mean annual temperature, where lighter shading (warmer colours) depict warmer areas. **(b)** Mean daily temperature for the sampled locations.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

713 **Table 1** Testing for differences in among-population variation across geography both within and across
714 temperature treatments. **(a)** ANOVA summaries for testing for significant different in variation among
715 population for latitudinal versus Victorian populations (within each treatment temperature). **(b)** Effect
716 sizes for maximum differences among populations for latitudinal and Victorian populations, which were
717 calculated as the difference in marginal mean between populations with the largest and smallest values
718 for each trait (i.e., the range). **(c)** ANOVA summaries testing population×temperature interactions within
719 each geographic region. Parameters in bold are significant at p<0.05. Temp = treatment temperature, and
720 Pop = population.

(a)

		Cold (mins)		Heat (mins)		Desiccation (hrs)		Wing size (centroid)	
Temp.	***df***	χ^2	**P**	χ^2	**P**	χ^2	**P**	χ^2	**P**
13°C	1	0.005	0.942	1.150	0.284	1.594	0.207	9.518	**0.002**
25°C	1	0.778	0.378	1.481	0.224	0.456	0.499	20.822	**<0.001**
29°C	1	0.035	0.852	0.029	0.865	0.052	0.819	8.208	**0.004**

(b)

Temp.	**Geography**	**Range**	**Range**	**Range**	**Range**
13°C	Latitude	4.12	1.72	1.03	0.18
	Victoria	4.79	3.13	2.91	0.05
25°C	Latitude	4.63	12.32	2.03	0.14
	Victoria	3.90	9.46	3.17	0.02
29°C	Latitude	10.46	10.48	2.37	0.12
	Victoria	11.03	11.78	2.93	0.04

(c)

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

721

722

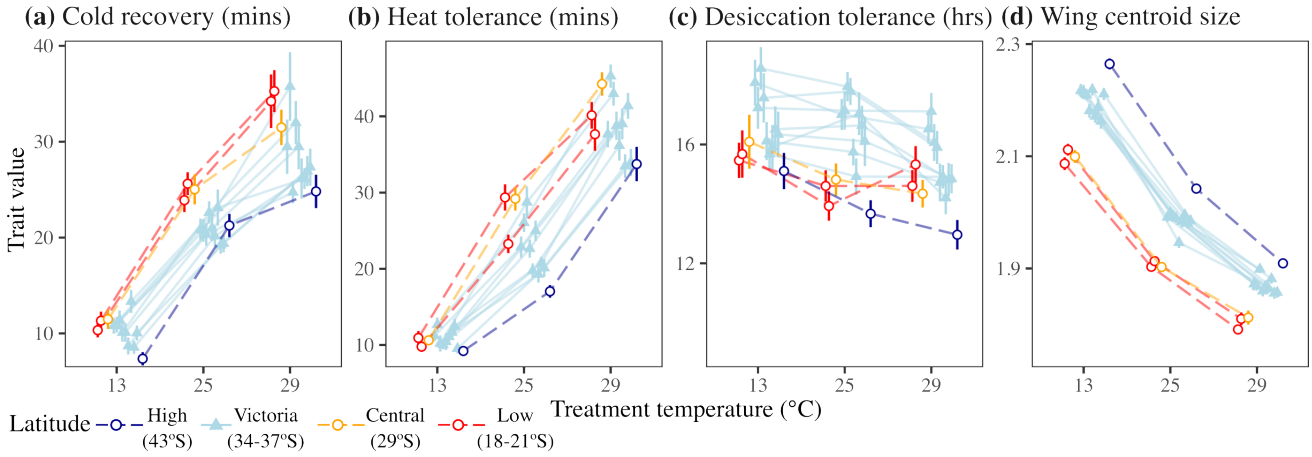

Fig. 2 The effect of treatment temperature on stress tolerance and body size for populations along latitude (open circles) and within Victoria (closed circles). We measured **(a)** cold tolerance as chill coma recovery time (mins), **(b)** heat tolerance as heat knockdown time (mins), **(c)** desiccation 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.

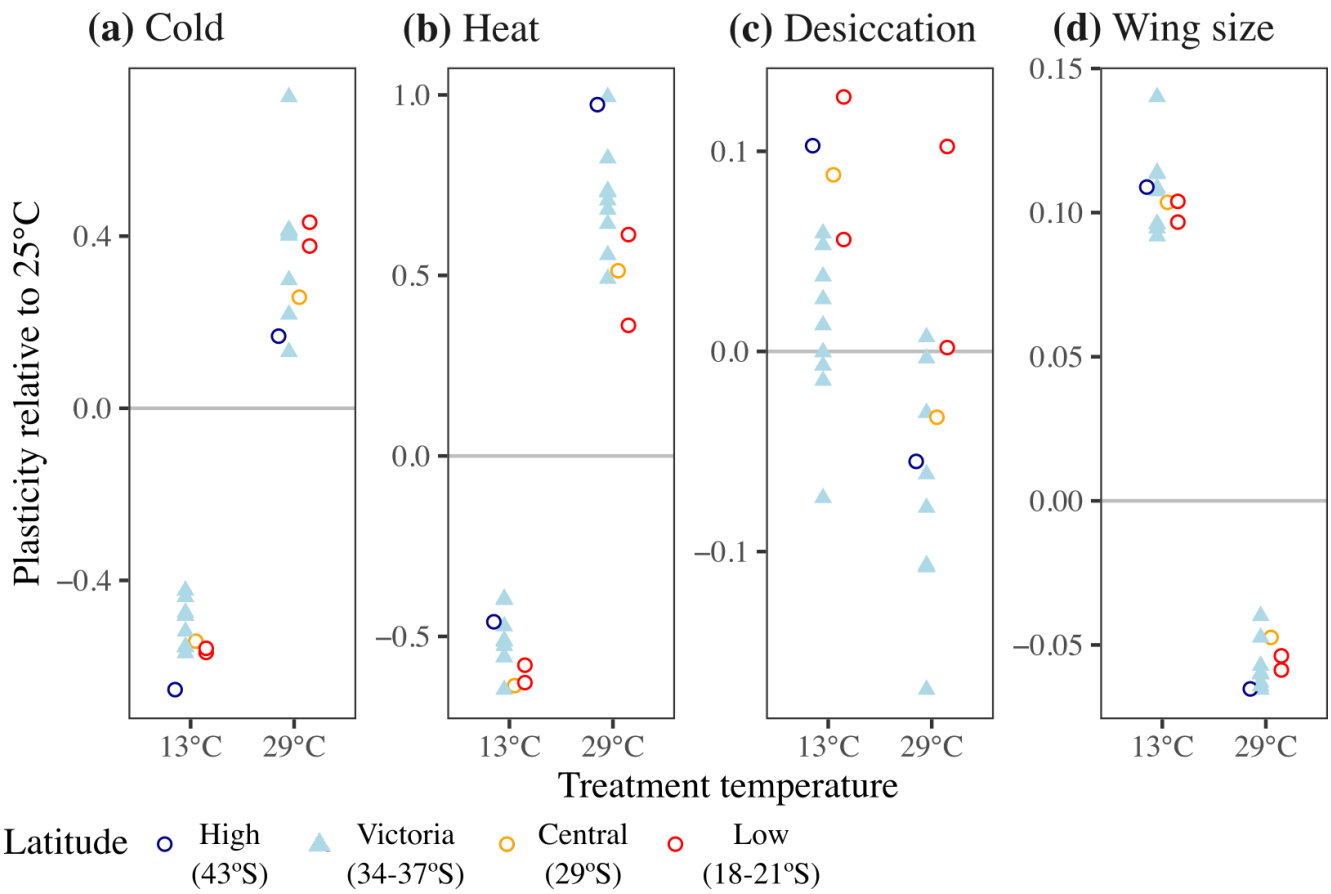

Fig. 3 Population variation in thermal plasticity for each trait (**a-d**). Triangles represent Victorian populations, and circles the latitudinal populations. Horizontal grey lines represent the control conditions (25°C), and the points represent the plasticity for each population as the change in phenotype from the control condition. Negative and positive values then represent a trait decrease and increase, respectively, relative to the 25°C treatment. Data points are offset for clarity.

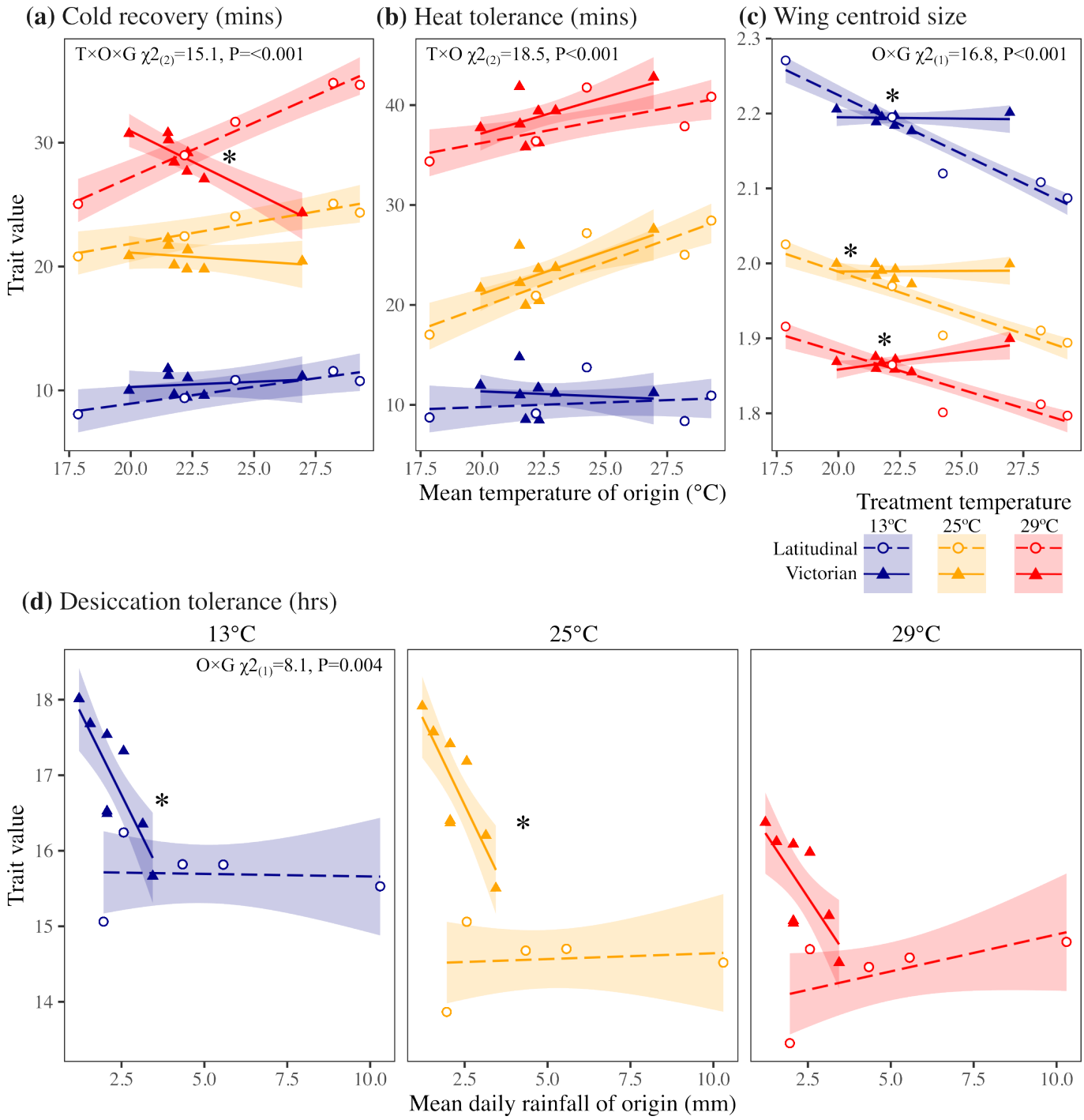

Fig. 4 Association between climate of origin and each trait (**a–d**) after exposure to three temperatures (different colours). Panels **a–c** compare mean daily temperature to cold tolerance, heat tolerance and wing size, while panel **d** compares average daily rainfall to desiccation tolerance. In panel **d**, treatment temperatures are presented in different panels to aid interpretation as they otherwise overlap substantially. Shading represents ±1SE around the regression estimates. 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. Asterisks represent significant differences in slopes between Victorian and latitudinal populations.

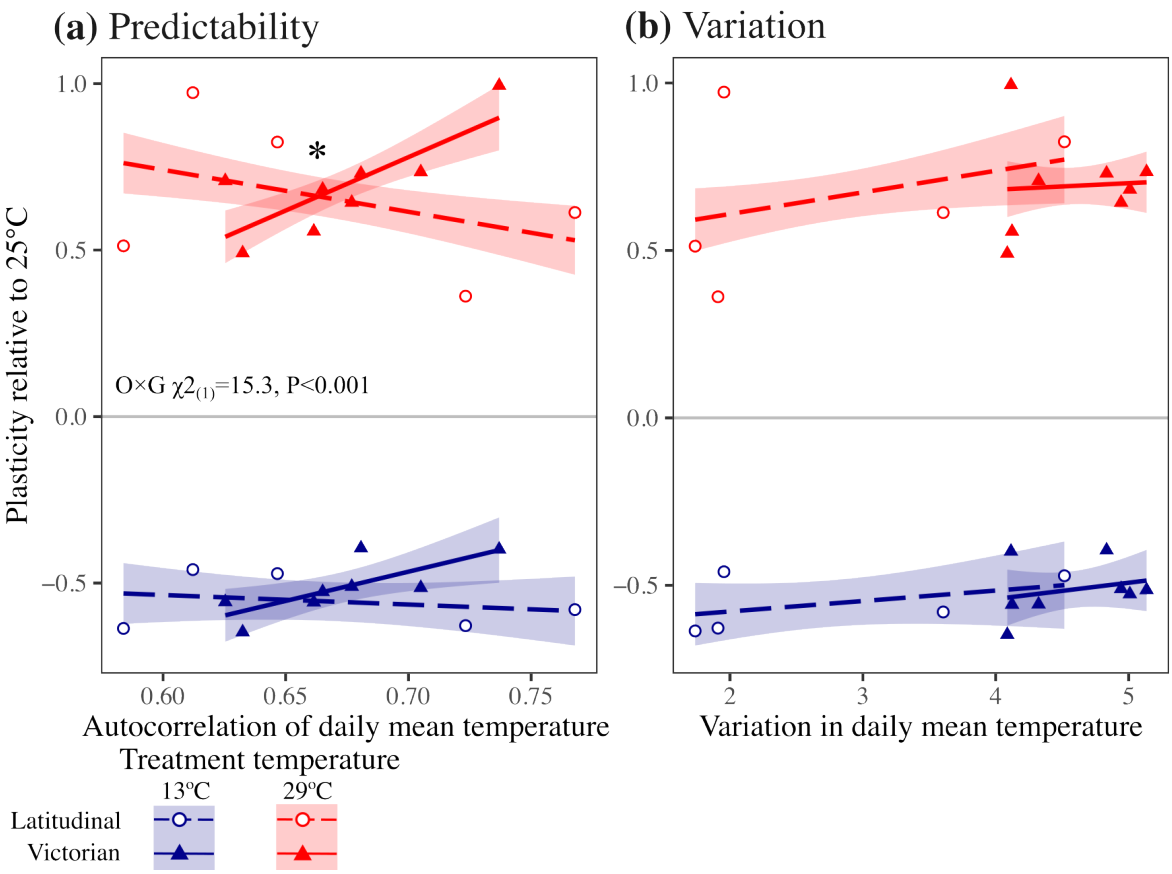

Fig. 5 Association between **(a)** predictability and **(b)** variation of daily mean temperature across three months, with plasticity in heat tolerance. Temperature predictability is the autocorrelation of mean daily temperature across the three months preceding sampling, while temperature variation was calculated as the standard deviation of temperature across the three months. Shading represents ±1SE around the regression estimates. 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 summaries. Asterisks represent significant differences in slopes between Victorian and latitudinal populations.