Variation in temperature but not diet determines the stability of latitudinal clines in tolerance traits and their plasticity

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

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

* Corresponding Author: Greg M. Walter
Email: greg.walter@utas.edu.au

ABSTRACT

Latitudinal clines in thermal tolerance are routinely used as evidence of adaptation across a broad climatic gradient. However, if the strength of latitudinal clines changes with the environment, then patterns of adaptation will be unstable, and using clinal patterns to predict population responses to global change will be difficult. To test whether changes in temperature and diet influence the strength of latitudinal patterns in key traits, we sampled five populations of *Drosophila melanogaster* spanning 3000-km of east coast Australia, and measured stress tolerance (heat, cold and desiccation) and body size on flies that developed in six combinations of temperature (13°C, 25°C and 29°C) and diet (standard and low-calorie) treatments. We found evidence of latitudinal clines whereby populations nearer the equator had smaller wings, lower cold tolerance and greater heat tolerance. For all traits, developmental temperature determined the strength of the latitudinal cline, whereas diet had little influence. Latitudinal clines were steeper in warmer treatments, which was created by latitudinal variation in plasticity. Compared to populations from higher latitudes, lower latitude populations that developed at the warmest temperature showed smaller increases in heat tolerance, greater decreases in cold tolerance, but a better ability to maintain desiccation resistance. These results suggest that latitudinal trends in plasticity underlie environment-dependent latitudinal clines, which are likely to determine variation in population responses to global change.

Keywords: body size, desiccation resistance, developmental plasticity, *Drosophila*, geographic variation, intraspecific variation, latitudinal cline, plasticity, thermal tolerance

Introduction

Determining how natural populations adapt to environmental variation across geography is crucial if we are to accurately predict their response to ongoing global change (Hoffmann & Sgrò 2011; Sgrò *et al.* 2011; Valladares *et al.* 2014). Latitudinal clines in ecologically important traits emerge in response to local adaptation across large climatic gradients (Huey *et al.* 2000; Hoffmann *et al.* 2002; Etterson 2004; Trotta *et al.* 2006; Arthur *et al.* 2008; Hopkins *et al.* 2008; Hernández *et al.* 2019; Lush *et al.* 2024), and are widely used to predict population responses to future environmental change (Hoffmann & Sgrò 2011; Sunday *et al.* 2011; Mayekar *et al.* 2022). However, current predictions of population responses to global change often assume that latitudinal patterns of adaptation are stable. For example, populations from warmer environments that have evolved higher heat tolerance are generally expected to respond better to warmer conditions created by climate change (Lush *et al.* 2024). If, however, populations along latitude vary in their response to environmental variation, then predicting population responses to global change will need to consider how populations vary in their sensitivity to the environment (Chevin *et al.* 2013; Valladares *et al.* 2014; Matesanz & Ramírez-Valiente 2019; Walter *et al.* 2020). While studies have emphasised the importance of incorporating plasticity and local adaptation in predictive models to increase the accuracy of predictions under climate change (Chevin *et al.* 2010; Reed *et al.* 2011; Bennett *et al.* 2019; Meek *et al.* 2023), we still lack a fundamental understanding of how patterns of adaptation change in response to environmental variation.

Latitudinal clines are typically estimated under a single temperature in common garden conditions, meaning that they rarely consider whether latitudinal patterns remain stable as the environment changes (Liefting *et al.* 2009). Testing whether patterns of adaptation, such as along a latitudinal gradient, remain stable across environments can reveal the extent to which locally adapted populations are likely to vary in their response to global change (DeMarche *et al.* 2018, 2019). While latitudinal clines in climatic stress tolerance traits can remain stable across sexes (Lasne *et al.* 2018) and simulated seasonal changes (Lush *et al.* 2024), pathogen infection can erase latitudinal clines in thermal tolerance (Hector *et al.* 2020), and patterns of climatic adaptation across latitudinal extremes can change with temperature (Liefting *et al.* 2009; van Heerwaarden & Sgrò 2011) and combinations of temperature and diet (Alton *et al.* 2020; Chakraborty *et al.* 2020). This suggests that developmental environment could determine the strength of latitudinal clines that represent adaptation across a broad climatic gradient. However, we lack an understanding of how combinations of environmental stressors during development determine the stability of latitudinal clines in climate tolerance, which limits our ability to accurately predict population responses to climate change (Louthan *et al.* 2021; Zettlemoyer 2023).

The capacity for individuals to adjust their phenotype to cope with environmental variation, known as phenotypic plasticity, is expected to help populations to cope with the environmental variation created by climate change (Via *et al.* 1995; Ghalambor *et al.* 2007; Charmantier *et al.* 2008; Reed *et al.* 2011; Morris

2014; Anderson & Gezon 2015; Acasuso-Rivero *et al.* 2019). Adaptation across latitude is not only expected to be associated with clinal patterns in trait means, but also variation in those traits that represent latitudinal differences in plasticity (Liefting *et al.* 2009; Molina-Montenegro & Naya 2012). If populations vary randomly in plasticity, then it will be difficult to predict the response of locally adapted populations to global change. While we know that adaptation to different environments can create changes in plasticity (Murren *et al.* 2014; Matesanz & Ramírez-Valiente 2019; Walter *et al.* 2022), we have a poor understanding of how populations adapted to a latitudinal gradient vary in plasticity.

Greater temperature heterogeneity at higher latitudes is expected to create greater thermal plasticity compared to more homogeneous temperature variation at lower latitudes (Janzen 1967; Ghalambor *et al.* 2006). This pattern of greater plasticity at higher latitudes has been found for a variety of fitness and foliar traits in plants (Molina-Montenegro & Naya 2012; Ren *et al.* 2020), and ectotherm species from higher latitudes show greater capacity to thermally acclimate (Seebacher *et al.* 2015; Kellermann & van Heerwaarden 2019) and have broader thermal tolerances (Sunday *et al.* 2011). However, these studies focussed on differences among species, which are less informative about how populations within a species will respond to climate change. Apart from two studies with *Drosophila* and *Sepsis* flies that found populations from latitudinal extremes showed differences in plasticity (Berger *et al.* 2013; van Heerwaarden & Sgrò 2017), we lack evidence that local adaptation (within a species) to latitude creates gradients in plasticity for thermal tolerance traits. Further manipulative experiments are therefore required to test whether adaptation across latitude favours clinal patterns in thermal plasticity, which will help to more accurately predict how populations from across a broad thermal gradient will respond to global change (Kellermann & van Heerwaarden 2019).

To test whether latitudinal clines in tolerance traits remain stable under varying developmental environments, we studied populations from a well-established latitudinal cline of *Drosophila melanogaster* along east-coast Australia (Hoffmann *et al.* 2002; Hoffmann & Weeks 2007; Sgrò *et al.* 2010). We used a manipulative laboratory experiment to test how clinal patterns in body size and tolerance traits, and their plasticity, changed across combinations of diet and temperature treatments. We sampled five populations of *D. melanogaster* across a 3000-km latitudinal cline in eastern Australia from Tasmania (43°S) to North Queensland (18°S; **Fig. 1**). In the lab, we reared larvae from each population in six treatments (three temperatures × two diets), and measured tolerance traits (heat, cold and desiccation) and wing size on the adults that emerged. We tested whether combinations of temperature and diet during development determined the strength and directionality of latitudinal clines in trait means and their plasticity (estimated across developmental treatments).

Methods

Collection and maintenance of experimental populations

Between March and May 2021, we collected 20-50 field-inseminated female *Drosophila melanogaster* using sweep nets over fruit waste from fruit farms (primarily banana and apple) at five locations across Tasmania, New South Wales, Victoria, and Northern Queensland (**Fig. 1**; **Table S1**). In the laboratory, we established isofemale lines by allowing the collected females to lay eggs for four days in 40mL vials containing 7mL of a standard yeast-potato-dextrose medium (potato flakes 18.2g/L; dextrose 27.3g/L; Brewer's yeast 36.4g/L; agar 6.4g/L, Nipagin [10% w/v in ethanol] 10.9mL/L; and propionic acid 4.6mL/L). The larvae were reared at 25°C on a 12:12 h light:dark cycle. In the subsequent generation, we verified species identification to avoid 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 eliminate differences in endosymbionts, particularly *Wolbachia*, which is more common in tropical populations. After three generations, we combined five males with five virgin females from each isofemale line (19-47 lines per population, mean=35.8; **Table S1**) into a mass-bred population maintained in five 300mL bottles containing 62.5mL of standard food. Each generation, adults were collected and kept in bottles until they reached peak fertility (approximately four days), after which they were allowed to lay 250-300 eggs in each of the five bottles. After laying, adults were removed, pupation cards were added, and larvae were left to develop. We maintained the populations at 25°C for 12 generations (approximately nine months) to ensure sufficient mixing of genotypes and to avoid conducting experiments during the 2021 COVID-19 lockdowns in Melbourne.

Lab experiment manipulating development temperature and diet

In January 2022, we conducted a manipulative laboratory experiment with the five mass-bred populations to test how combined thermal and nutritional stress influences clinal patterns in tolerance traits and body size. We picked eggs from all populations into six treatments, which included three constant temperatures (13, 25 and 29°C) and two diets (standard diet and a low-calorie 25% dilution diet). The 25°C condition served as the control, while 13°C and 29°C represented the cold and hot extremes that larvae encounter in natural populations during winter and summer, respectively. The low-calorie diet was chosen to simulate reduced food availability predicted under climate change (Chakraborty et al. 2023). For each population, approximately 200 adult flies were placed in two laying pots with a thin layer of food at the bottom and allowed to lay eggs for six hours. We then transferred 20 eggs into each vial (n=36-42 vials/population/treatment; **Table S2**) containing 7mL of either standard or low-calorie (25% dilution) food and allowed larvae to develop at their respective treatment temperatures. To manage replication, the experiment was divided into two blocks, with adults emerging between January 21-28 (block 1) and March 1-8 (block 2).

138 For each block, we conducted 2-3 rounds of egg-picking for each treatment over 4-5 days to maximize
139 overlap in eclosion across treatments (n=14-21 vials/population/treatment/block). We altered egg-picking
140 times based on pilot experiments to ensure that adults would emerge simultaneously so that they could be
141 randomised into the same thermal tolerance assays (**Table S2**). We then placed eggs into 36-42 vials per
142 population per treatment combination, split across 5-6 rounds of egg-picking over the two experimental
143 blocks (**Table S2**). In total, 2912 vials were used (n=455-546 vials per treatment).

144 When adults started eclosing, we collected them daily in separate vials for each treatment to ensure
145 uniform age across treatments. We then kept flies at their experimental conditions until they matured and
146 mated. In pilot experiments, we found that flies emerging at 13°C would not mature if kept at 13°C, so
147 those collected from 13°C were kept at 16°C to mature in time for the experiment (seven days until egg-
148 laying).

149

150 *Tolerance assays*
151 We measured females as their body size and thermal tolerance are more sensitive to nutritional and
152 temperature variation (Kutz et al. 2019; Chakraborty et al. 2020), and because sex-specific clinal trends
153 were not the focus of our study. We collected females using light CO_2 anesthesia, allowed them to
154 recover for 48 hours, and then assayed them for cold, heat and desiccation tolerance following established
155 methods (Hoffmann et al. 2002; Lasne et al. 2018). For each tolerance trait, we measured 30 females per
156 population per treatment combination. For heat tolerance, we measured heat knockdown by placing
157 individual females in sealed 5mL glass vials that we submerged in a 39°C water bath and then recorded
158 the time it took each female to cease moving. For cold tolerance, we measured chill-coma recovery by
159 placing individual females in sealed 1.5mL plastic tubes submerged in a 0°C water bath for four hours
160 and then recorded the time they took to stand up. For desiccation tolerance, we placed individual females
161 in 5mL glass vials covered with mesh for airflow, which we placed within sealed tanks containing silica
162 gel that maintained <5% relative humidity, and recorded the time to cessation of movement. To measure
163 body size, we quantified wing size by mounting the right wing of 30 females in SH solution (70%
164 ethanol, 30% glycerol) on microscope slides, photographing them using a Leica M60 stereo microscope
165 (Leica, Heerbrugg, Switzerland), and using ImageJ to measure 10 vein intersection landmarks (**Fig. S1**),
166 which we used to estimate wing centroid size.

167 *Statistical analyses*
168 We used *R* (v.4.4.1) statistical software for all analyses (R Core Team 2024). Using the package
169 *glmmTMB* (Brooks *et al.* 2017), we implemented linear mixed models to test for clinal patterns in the four
170 traits (thermal and desiccation tolerance, and wing size), and whether these patterns changed depending
171 on developmental temperature and diet. We checked model assumptions using *DHARMa* (Hartig 2022).

We tested each trait independently and included latitude (continuous trait) and developmental temperature and diet as the fixed effects, as well as their three-way interaction. We also included block as a fixed effect and assay run (within block) as the only random effect. We then used *emmeans* (Lenth 2016) to obtain the marginal means for each population under each temperature and diet combination. While a significant latitude term would provide evidence for a latitudinal trend in a given trait, significant treatment × latitude terms would suggest that the clinal trends change depending on the developmental environment.

To test for clinal patterns in plasticity, we used the marginal means to quantify plasticity as the change in trait value from the control temperature (25°C). We calculated plasticity separately for the two diets, meaning that we focus on plasticity in response to temperature. This was because we found stronger effects of temperature than diet, but the results did not change when we used only the 25°C, 100% diet as the control treatment. We then used a linear model to test for significant clinal trends in plasticity, and significant effects of developmental temperature on clinal patterns.

Results

Clinal patterns in tolerance traits and body size change more with temperature than diet

We found evidence of latitudinal clines in all traits. At the control temperature (25°C), higher latitudes showed higher cold tolerance, lower heat tolerance, higher desiccation resistance and larger wing (body) size (**Fig. 2**). We found no significant diet×temperature×latitude interactions for any trait (**Table 1**), suggesting that latitudinal trends did not change across all combinations of diet and temperature. Diet×latitude was only significant for cold tolerance, whereas temperature×latitude interactions were significant for all traits (**Table 1**). The strength of clinal patterns therefore depended more on developmental temperature than diet.

For cold tolerance, latitudinal clines were twice as strong at the standard diet (marginal slope=-0.22±0.04) compared to the low-calorie diet (marginal slope=-0.09±0.04), with the strongest clinal pattern present at the warmest developmental treatment (**Fig. 2a; Table S3**). For heat tolerance, we found the strongest latitudinal cline at the control temperature (marginal slope for 25°C=-0.38±0.05), with a weaker clinal pattern at the warmest development temperature (marginal slope for 29°C=-0.18±0.05) and no latitudinal cline at the coldest temperature (marginal slope for 13°C=-0.02±0.05; **Fig. 2b; Table S3**). Desiccation resistance showed weak positive trends across latitude for the cold and control developmental temperatures (marginal slope for 13°C=0.01±0.02, 25°C=0.03±0.02), suggesting that higher latitudes showed slightly greater desiccation resistance. By contrast, desiccation resistance was greater at lower

latitudes in the warmest developmental temperature (marginal slope for 29°C=-0.05±0.02), suggesting that lower latitudes showed greater desiccation resistance after developing at a warm temperature (**Fig. 2c**). Body size increased at higher latitudes for all developmental treatments (**Fig. 2d**), with the coldest developmental treatment showing the strongest latitudinal cline (marginal slope for 13°C=0.006±0.0004) compared to the warmer temperatures (marginal slope for 25°C=0.005±0.0004, 29°C=0.005±0.0004).

Clinal patterns in plasticity are trait-specific and depend on developmental temperature

Only plasticity in heat tolerance and desiccation resistance showed significant latitudinal clines (**Fig. 3; Table 2**). For plasticity in heat tolerance, we found a significant latitude×temperature interaction (**Table 2b**), created by higher latitude populations showing greater increases in heat tolerance after developing at 29°C (marginal slope=0.018±0.003) compared to 13°C (marginal slope=0.006±0.003; **Fig. 3b**). This meant that higher latitude populations showed greater plasticity, seen as a greater increase in heat tolerance after developing at 29°C, and a smaller decrease in heat tolerance when larvae developed at 13°C (**Fig. 3b**). For desiccation resistance, populations from lower latitudes showed greater increases in desiccation resistance at all developmental treatments (**Fig. 3c**). By contrast, populations from higher latitudes showed lesser increases in desiccation resistance when larvae developed at 13°C (marginal slope=-0.002±0.002), and a reduction in desiccation resistance when they developed at 29°C (marginal slope=-0.007±0.002; **Fig. 3c**). Plasticity in body size and cold tolerance showed no significant latitudinal trends.

Discussion

Studies of latitudinal clines have demonstrated adaptation across broad climate gradients, but rarely do they consider whether patterns of adaptation remain stable across environments. Here, we tested how the strength of latitudinal clines in tolerance traits and their plasticity changed across combinations of development diet and temperature. We found that temperature, but not diet (except for cold tolerance), influenced the strength of clinal patterns in all traits (**Fig. 2**). We also found that developmental temperature determined the strength of latitudinal clines for plasticity in heat tolerance and desiccation resistance (**Fig. 3b-c**). Stronger latitudinal trends emerged when flies developed in warmer temperatures, whereas stronger clines in body size emerged in the coldest development temperature. Latitudinal clines generally followed previously reported patterns: populations from lower latitudes showed lower cold tolerance, higher heat tolerance and smaller wing sizes, but surprisingly, higher desiccation tolerance. For plasticity, higher latitude populations showed greater plasticity as a greater increase in heat tolerance and as a stronger reduction in desiccation tolerance. These results provide evidence that the strength of

latitudinal clines is sensitive to variation in temperature, and that environmental change is likely to affect populations adapted across latitude differently due to variation in the capacity for populations to respond via phenotypic plasticity.

Variation in temperature changes latitudinal clines more than diet

Developmental temperature created variation in the strength of latitudinal clines for all traits, whereas the dilution diet had little effect on the strength of latitudinal clines. This suggests that changes in the availability of food are less likely to override signals of climatic adaptation. However, a study that manipulated the protein and carbohydrate availability in diet treatments for three populations of *D. melanogaster* along a latitudinal cline found significant interactions between diet, temperature and population on body size and development time. These results suggested that populations varied in plasticity in response to developmental environment, but in a way that was idiosyncratic for populations across latitude (Chakraborty *et al.* 2020). This finding of idiosyncratic plasticity is consistent with another study showing that the activation of the immune system in populations of *D. melanogaster* along a cline also produced idiosyncratic responses, which erased the latitudinal cline in heat tolerance (Hector *et al.* 2020). Given that food composition and availability is unlikely to show a similar latitudinal gradient to temperature, it is possible that adaptation to nutrition along the east coast of Australia will be region specific, creating patterns of local adaptation without a latitudinal cline.

The strength of latitudinal clines changed across developmental temperatures in a manner that was trait-dependent. For cold recovery, we observed the strongest latitudinal cline in the warmest temperature (**Fig. 2a**), which was because lower latitude populations took longer to recover from a chill coma after developing in a warm environment. Low latitude populations therefore struggled with the higher developmental temperature compared to higher latitude populations. This is consistent with evidence that populations from low latitudes are closer to their higher thermal limits and are likely to struggle with other stressors, such as rapid changes in temperature (Kellermann *et al.* 2012; van Heerwaarden *et al.* 2016). Body (wing) size showed the strongest latitudinal cline in the cold developmental temperature (**Fig. 2d**), suggesting that lower latitude populations increased their body size in cooler temperatures marginally more than the higher latitude populations (**Fig. 3d**). Both heat tolerance and desiccation resistance showed strong clinal patterns that changed with developmental temperature (**Fig. 2b-c**). Below we discuss the effects of plasticity on determining the strength of latitudinal clines in heat tolerance and desiccation resistance.

Linking the stability of latitudinal clines with plasticity

The latitudinal clines in plasticity were likely responsible for changes in the strength of clinal patterns that we observed across temperature, particularly for heat tolerance and desiccation resistance. While strong patterns of adaptation (i.e., strong latitudinal clines) were found at the control temperature (25°C) for heat tolerance (**Fig. 2b**), the weaker clinal trends found at the warmer developmental temperature highlights

that assessing clinal trends only under control temperatures could underestimate the potential for higher latitude populations to maintain resilience in response to ongoing climate change. This is because the high latitude populations showed the greatest plastic increase in heat tolerance when they developed at 29°C (**Fig. 3b**). Therefore, populations from higher latitudes showed lower heat tolerance, but greater plasticity in heat tolerance, suggesting a trade-off between heat tolerance and its plasticity. This supports recent work showing trade-offs between heat tolerance and its plasticity, which will be important to consider for predicting population responses to climate change (van Heerwaarden & Kellermann 2020; Bogan *et al.* 2024; van Heerwaarden *et al.* 2024).

Desiccation resistance showed zero or weak positive latitudinal clines in cold or control temperatures, but a strong negative latitudinal cline in the warmest developmental temperature (**Fig. 2c**). At 29°C, higher latitude populations were the least desiccation resistant under warm conditions, which was associated the greatest plastic decrease in desiccation resistance (**Fig. 3c**). This suggests that higher latitude populations struggle to maintain desiccation resistance in warmer developmental treatments. Given that higher latitude populations showed greater heat tolerance via plasticity under warmer temperatures, but reduced desiccation resistance, it is possible that locally adapted populations will show different trade-offs among traits that will determine responses to environmental change (Hoffmann 1990, 1991; Terblanche & Kleynhans 2009; Kellermann *et al.* 2020; van Heerwaarden & Kellermann 2020). These findings demonstrate that accurately predicting the response of populations to climate change requires considering how populations vary in their ability to respond via phenotypic plasticity across latitude, and how trade-offs among traits will affect population resilience.

Clines in plasticity support the climate variability hypothesis for heat tolerance

Our results support the theory that considering how locally adapted populations vary in plasticity will be key to accurately predicting their response to global change (Chevin *et al.* 2013; Valladares *et al.* 2014). As predicted by the climate variability hypothesis (Janzen 1967; Ghalambor *et al.* 2006; Ren *et al.* 2020), we found that higher latitude populations showed greater plasticity in heat tolerance (**Fig. 3b**). This suggests that higher plasticity in heat tolerance has evolved in higher latitude populations in response to greater variation in temperature compared to the more constant high temperatures present at low latitudes. By contrast, the clinal pattern for plasticity in desiccation resistance showed a surprising trend. While flies from lower latitudes consistently increased their desiccation resistance under stress, flies from higher latitudes showed drastic reductions in desiccation resistance when they developed in a hot temperature (**Fig. 3c**). This suggests that adapting to more constant high temperatures at low latitudes could preadapt populations for desiccation resistance at high temperatures. Determining how interactions among traits and their plasticity determines adaptive responses to climate change therefore remains an ongoing challenge for predicting the resilience of natural populations.

Conclusions

Our results reveal that predicting population responses to climate change based only under a single common garden condition can be inaccurate. The strength of latitudinal clines in tolerance traits and body size changed dramatically with developmental temperature, but was not strongly influenced by diet treatment. Accurately predicting population responses to climate change therefore requires understanding how patterns of adaptation emerge, and identifying which populations are likely to be able to persist under rapid global change.

Acknowledgements

We are grateful to Carmen da Silva, Julian Beaman and Belinda van Heerwaarden for helping to sample the populations. We also thank Emily Lombardi, Mia Wansbrough and Sandra Hangartner for their help with some of the assays. GMW was supported by Australian Research Council fellowships DE200101019 and FT240100466.

References

1.
Acasuso-Rivero, C., Murren, C.J., Schlichting, C.D. & Steiner, U.K. (2019). Adaptive phenotypic plasticity for life-history and less fitness-related traits. *Proc. R. Soc. Lond. B.*, 286.

2.
Alton, L.A., Kutz, T.C., Bywater, C.L., Beaman, J.E., Arnold, P.A., Mirth, C.K. *et al.* (2020). Developmental nutrition modulates metabolic responses to projected climate change. *Funct. Ecol.*, 34, 2488-2502.

3.
Anderson, J.T. & Gezon, Z.J. (2015). Plasticity in functional traits in the context of climate change: a case study of the subalpine forb *Boechera stricta* (Brassicaceae). *Global Change Biol.*, 21, 1689-1703.

4.
Arthur, A.L., Weeks, A.R. & Sgrò, C.M. (2008). Investigating latitudinal clines for life history and stress resistance traits in Drosophila simulans from eastern Australia. *J. Evol. Biol.*, 21, 1470-1479.

5.
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.

6.
Berger, D., Postma, E., Blanckenhorn, W.U. & Walters, R.J. (2013). Quantitative genetic divergence and standing genetic (co)variance in thermal reaction norms along latitude. *Evolution*, 67, 2385-2399.

7.
Bogan, S.N., Porat, O.I., Meneses, M.J. & Hofmann, G.E. (2024). Thermal plasticity has higher fitness costs among thermally tolerant genotypes of *Tigriopus californicus*. *Funct. Ecol.*, 38, 1562–1577.

8.
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.

9.
Chakraborty, A., Sgrò, C.M. & Mirth, C.K. (2020). Does local adaptation along a latitudinal cline shape plastic responses to combined thermal and nutritional stress? *Evolution*, 74, 2073-2087.

10.
Charmantier, A., McCleery, R.H., Cole, L.R., Perrins, C., Kruuk, L.E.B. & Sheldon, B.C. (2008). Adaptive phenotypic plasticity in response to climate change in a wild bird population. *Science*, 320, 800-803.

11.
Chevin, L.M., Collins, S. & Lefèvre, F. (2013). Phenotypic plasticity and evolutionary demographic responses to climate change: taking theory out to the field. *Funct. Ecol.*, 27, 966-979.

12.
Chevin, L.M., Lande, R. & Mace, G.M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.*, 8, e1000357.

13.
DeMarche, M.L., Doak, D.F. & Morris, W.F. (2018). Both life-history plasticity and local adaptation will shape range-wide responses to climate warming in the tundra plant *Silene acaulis*. *Global Change Biol.*, 24, 1614-1625.

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.
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.

16.
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.

17.
Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.*, 21, 394-407.

18.
Hartig, F. (2022). DHARMa: Residual diagnostics for hierarchical (multi-Level / mixed) regression models. R package.

19.
Hector, T.E., Sgrò, C.M. & Hall, M.D. (2020). The influence of immune activation on thermal tolerance along a latitudinal cline. *J. Evol. Biol.*, 33, 1224-1234.

20.
Hernández, F., Poverene, M., Garayalde, A. & Presotto, A. (2019). Re-establishment of latitudinal clines and local adaptation within the invaded area suggest rapid evolution of seed traits in Argentinean sunflower (*Helianthus annuus* L.). *Biol. Invasions*, 21, 2599-2612.

21.
Hoffmann, A.A. (1990). Acclimation for desiccation resistance in *Drosophila melanogaster* and the association between acclimation responses and genetic variation. *J. Insect Physiol.*, 36, 885-891.

22.
Hoffmann, A.A. (1991). Acclimation for desiccation resistance in Drosophila: Species and population comparisons. *J. Insect Physiol.*, 37, 757-762.

23.
Hoffmann, A.A., Anderson, A. & Hallas, R. (2002). Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecol. Lett.*, 5, 614-618.

24.
Hoffmann, A.A. & Sgrò, C.M. (2011). Climate change and evolutionary adaptation. *Nature*, 470, 479-485.

25.
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.

26.
Hopkins, R., Schmitt, J. & Stinchcombe, J.R. (2008). A latitudinal cline and response to vernalization in leaf angle and morphology in *Arabidopsis thaliana* (Brassicaceae). *New Phytol.*, 179, 155-164.

27.
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.

28.
Janzen, D.H. (1967). Why mountain passes are higher in the tropics. *Am. Nat.*, 101, 233-249.

29.
Kellermann, V., McEvey, S.F., Sgrò, C.M. & Hoffmann, A.A. (2020). Phenotypic plasticity for desiccation resistance, climate change, and future species distributions: Will plasticity have much impact? *Am. Nat.*, 196, 306-315.

30.
Kellermann, V., Overgaard, J., Hoffmann, A., Flogaard, C., Svenning, J.-C. & Loeschcke, V. (2012). Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. *Proc. Natl. Acad. Sci. USA*, 109, 16228-16233.

31.
Kellermann, V. & van Heerwaarden, B. (2019). Terrestrial insects and climate change: adaptive responses in key traits. *Physiol. Entomol.*, 44, 99-115.

32.
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.

33.
Lenth, R.V. (2016). Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, 69, 1-33.

34.
Liefting, M., Hoffmann, A.A. & Ellers, J. (2009). Plasticity versus environmental canalization: population differences in thermal responses along a latitudinal gradient in *Drosophila serrata*. *Evolution*, 63, 1954-1963.

35.
Louthan, A.M., DeMarche, M.L. & Shoemaker, L.G. (2021). Climate sensitivity across latitude: scaling physiology to communities. *Trends Ecol. Evol.*, 36, 931-942.

36.
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*.

37.
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.

38.
Mayekar, H.V., Ramkumar, D.K., Garg, D., Nair, A., Khandelwal, A., Joshi, K. *et al.* (2022). Clinal variation as a tool to understand climate change. *Frontiers in Physiology*, 13.

39.
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.

40.
Molina-Montenegro, M.A. & Naya, D.E. (2012). Latitudinal patterns in phenotypic plasticity and fitness-related traits: assessing the climatic variability hypothesis (CVH) with an invasive plant species. *PLoS One*, 7, e47620.

41.
Morris, M.R. (2014). Plasticity-mediated persistence in new and changing environments. *International Journal of Evolutionary Biology*, 2014, 416497.

42.
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.

43.
R Core Team (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna, Austria.

44.
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.

45.
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.

46.
Seebacher, F., White, C.R. & Franklin, C.E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, 5, 61-66.

47.
Sgrò, C.M., Lowe, A.J. & Hoffmann, A.A. (2011). Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications*, 4, 326-337.

48.
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.

49.
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.

50.
Terblanche, J.S. & Kleynhans, E. (2009). Phenotypic plasticity of desiccation resistance in Glossina puparia: are there ecotype constraints on acclimation responses? *J. Evol. Biol.*, 22, 1636-1648.

51.
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.

52.
Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M.B., Balaguer, L., Benito-Garzón, M. *et al.* (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecol. Lett.*, 17, 1351-1364.

53.
van Heerwaarden, B. & Kellermann, V. (2020). Does plasticity trade off with basal heat tolerance? *Trends Ecol. Evol.*, 35, 874-885.

54.
van Heerwaarden, B., Kellermann, V. & Sgrò, C.M. (2016). Limited scope for plasticity to increase upper thermal limits. *Funct. Ecol.*, 30, 1947-1956.

55.
van Heerwaarden, B., Sgrò, C. & Kellermann, V.M. (2024). Threshold shifts and developmental temperature impact trade-offs between tolerance and plasticity. *Proc. R. Soc. Lond. B.*, 291, 20232700.

56.
van Heerwaarden, B. & Sgrò, C.M. (2011). The effect of developmental temperature on the genetic architecture underlying size and thermal clines in *Drosophila melanogaster* and *D. simulans* from the east coast of Australia. *Evolution*, 65, 1048-1067.

57.
van Heerwaarden, B. & Sgrò, C.M. (2017). The quantitative genetic basis of clinal divergence in phenotypic plasticity. *Evolution*, 71, 2618-2633.

58.
Via, S., Gomulkiewicz, R., de Jong, G., Scheiner, S.M., Schlichting, C.D. & Van Tienderen, P.H. (1995). Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol. Evol.*, 10, 212-217.

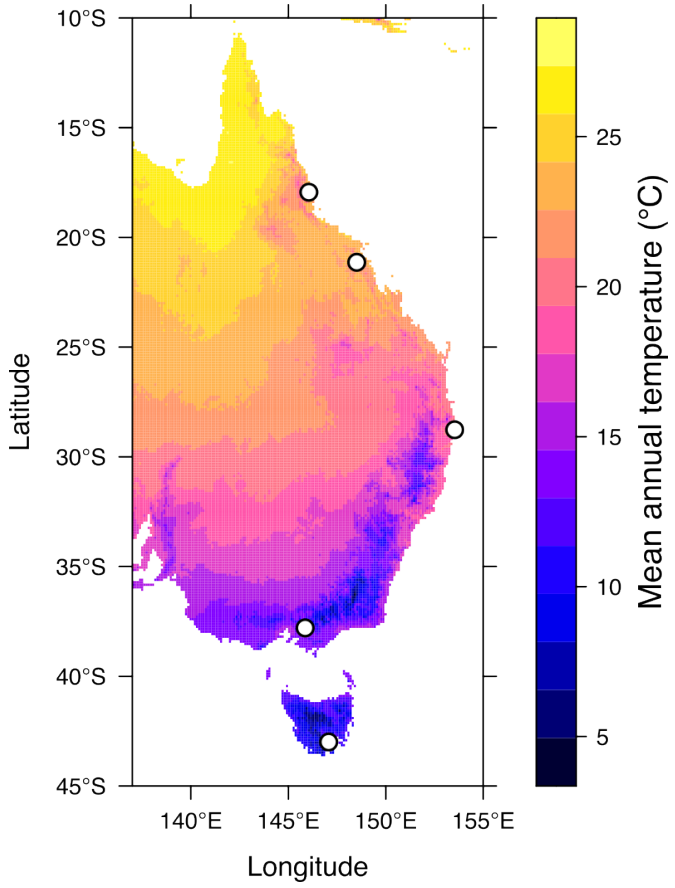
59.
Walter, G.M., Catara, S., Bridle, J.R. & Cristaudo, A. (2020). Population variation in early development can determine ecological resilience in response to environmental change. *New Phytol.*, 226, 1312-1324.

60.
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.

61.
Zettlemoyer, M.A. (2023). A demographic approach for predicting population responses to multifactorial stressors. *AoB Plants*, 15, plad023.

530 **Tables & Figures**

531



532

533 **Fig. 1** Map of sampling locations along the cline (n=5; unfilled circles). Colour represents the average
534 mean annual temperature.

535

536 **Table 1** ANOVA summaries for testing the stability of latitudinal clines for the four traits across
537 developmental diets and temperatures. Terms that are significant at P<0.05 are denoted in bold.

		(a) Cold tolerance		**(b)** Heat tolerance		**(c)** Desiccation resistance		**(d)** Wing centroid size	
Term	Df	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Intercept	1	875.4	**<0.001**	1030.1	**<0.001**	1510.6	**<0.001**	63836.1	**<0.001**
Latitude	1	31.8	**<0.001**	52.5	**<0.001**	0.1	0.721	568.9	**<0.001**
Temperature	2	161.0	**<0.001**	261.0	**<0.001**	9.0	**0.011**	221.6	**<0.001**
Diet	1	7.5	**0.006**	0.1	0.748	34.0	**<0.001**	2.5	0.113
Block	1	33.9	**<0.001**	1.1	0.301	4.2	**0.041**	-	
Latitude × Temperature	2	9.5	**0.009**	30.2	**<0.001**	10.8	**0.005**	10.7	**0.005**
Latitude × Diet	1	5.3	**0.022**	1.2	0.283	2.2	0.140	0.1	0.712
Temperature × Diet	2	1.8	0.405	0.5	0.794	0.7	0.701	2.7	0.264
Latitude × Temperature × Diet	2	1.6	0.450	0.1	0.970	0.6	0.748	4.7	0.097

538

539

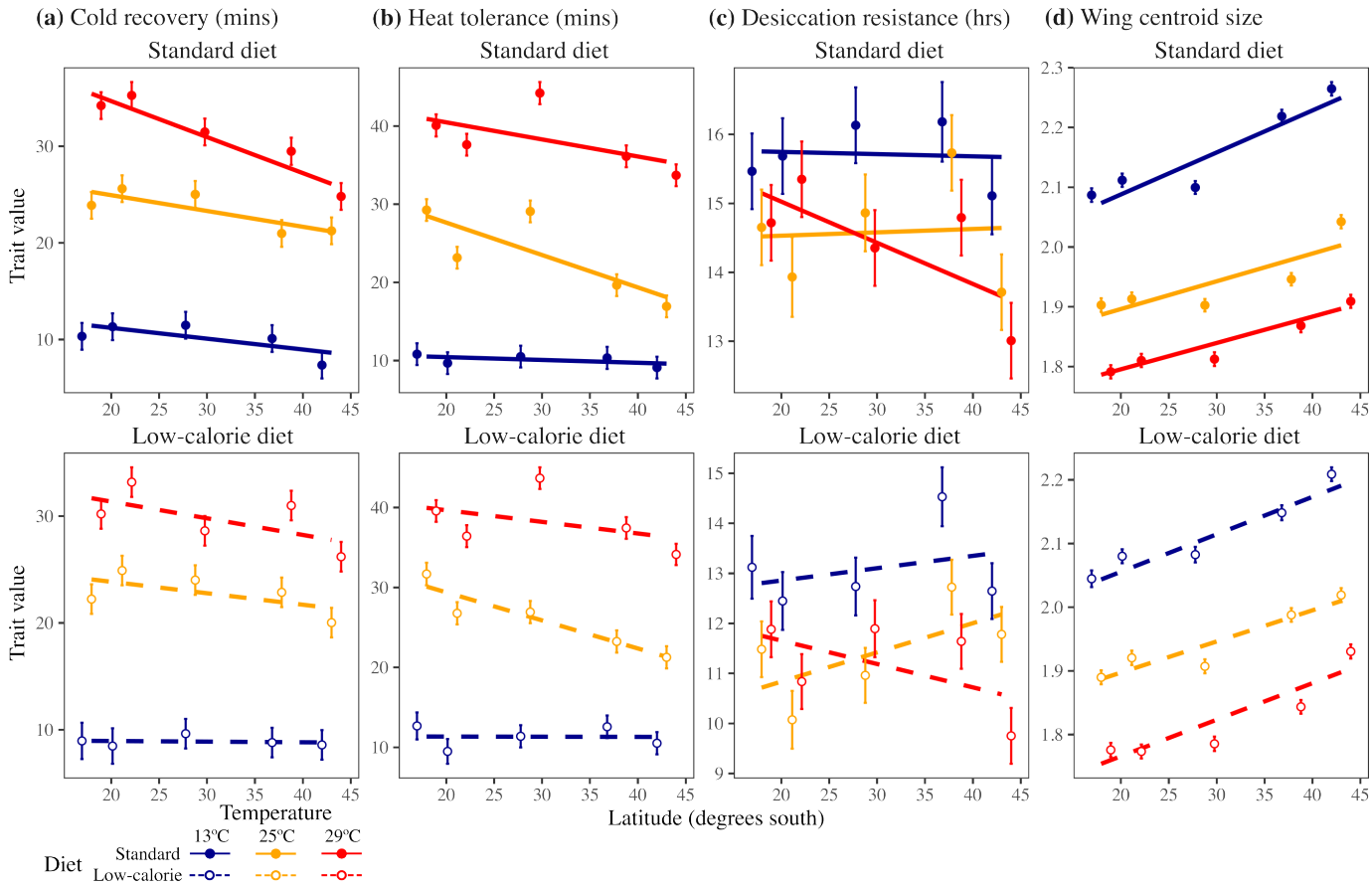


Fig. 2 Testing for latitudinal clines in **(a)** cold tolerance, **(b)** heat tolerance, **(c)** desiccation resistance, and **(d)** wing size. Circles and credible intervals represent the mean (±1SE). Colours depict the different temperature treatments. Closed circles and solid lines represent the standard diet (upper panels), open circles and dashed lines the low-calorie diet (lower panels).

546 **Table 2** ANOVA summaries for testing the stability of latitudinal clines in plasticity across
547 developmental temperatures (i.e., plastic change in trait from control 25°C to each other temperature).
548 Values above and below zero denote plasticity from the control as an increase and decrease, respectively,
549 in that trait. Terms that are significant at P<0.05 are denoted in bold.

		(a) Cold tolerance			**(b)** Heat tolerance			**(c)** Desiccation resistance			**(d)** Wing centroid size		
Term	Df	SS	F	P	SS	F	P	SS	F	P	SS	F	P
Intercept	1	0.01	2.24	0.161	0.22	31.76	**<0.001**	0.06	22.88	**<0.001**	0.00	0.51	0.490
Latitude	1	0.01	1.37	0.264	0.26	37.27	**<0.001**	0.04	12.97	**0.004**	0.00	2.75	0.123
Temperature	1	0.48	96.44	**<0.001**	0.30	42.71	**<0.001**	0.00	0.02	0.883	0.01	69.89	**<0.001**
Diet	1	0.01	2.79	0.121	0.00	0.06	0.807	0.01	3.74	0.077	0.00	0.77	0.399
Latitude × Temperature	1	0.01	1.19	0.298	0.06	8.07	**0.015**	0.01	3.44	0.088	0.00	0.34	0.568
Latitude × Diet	1	0.01	2.11	0.172	0.01	1.06	0.324	0.01	2.49	0.141	0.00	0.01	0.908
Temperature × Diet	1	0.00	0.07	0.794	0.00	0.01	0.941	0.00	0.07	0.800	0.00	0.43	0.526
Latitude × Temperature × Diet	1	0.00	0.15	0.701	0.01	0.71	0.415	0.00	0.06	0.812	0.00	1.21	0.293
Residual	12	0.06			0.08			0.03			0.00		

550

551

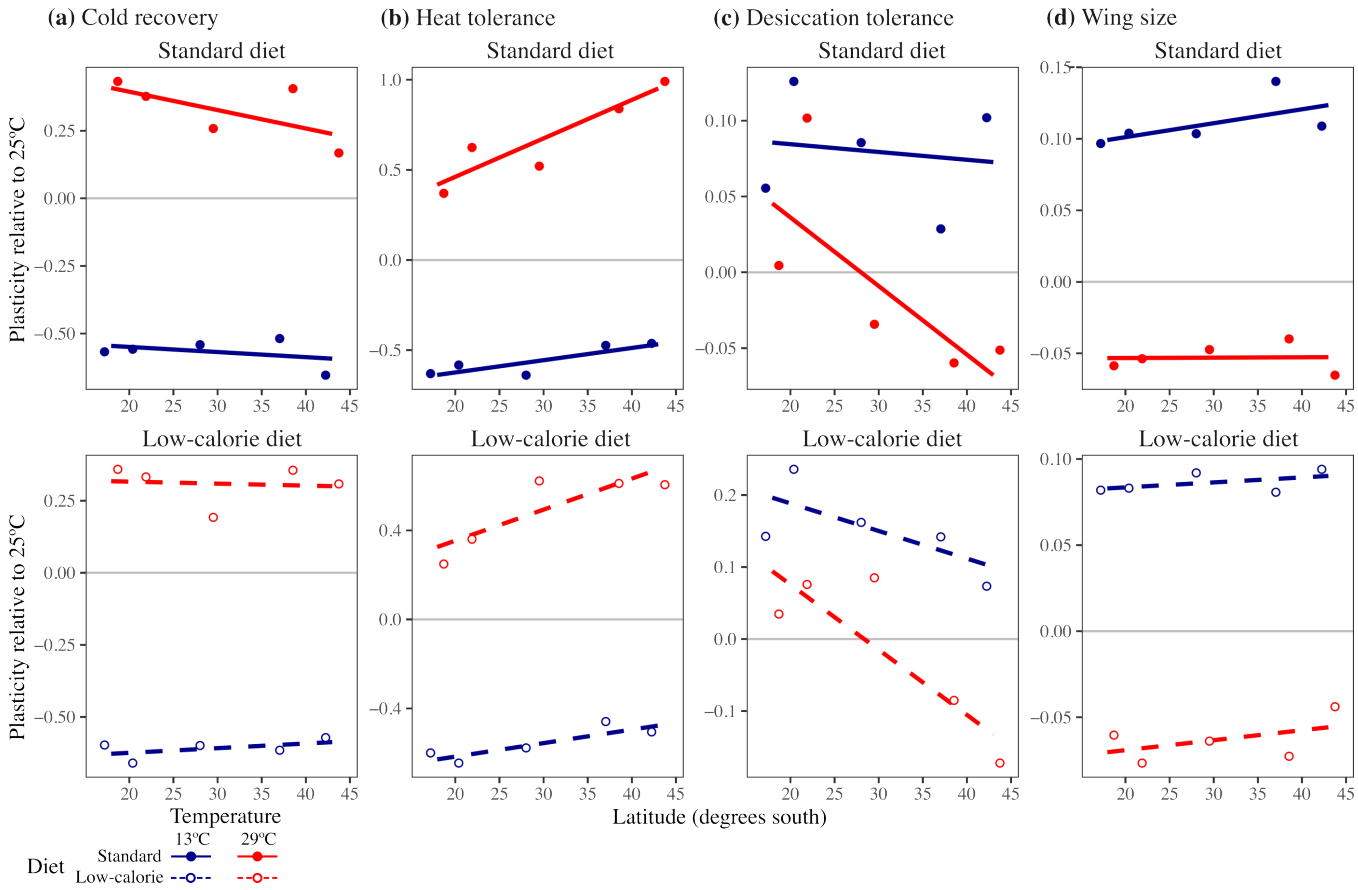


Fig. 3 Testing for latitudinal clines in plasticity for **(a)** cold recovery, **(b)** heat tolerance, **(c)** desiccation tolerance, and **(d)** wing size. Colours depict the different developmental temperatures. Closed circles and solid lines represent the standard diet (upper panels), open circles and dashed lines the low-calorie diet (lower panels). Plasticity values above and below zero increase and decrease the trait from the control condition, respectively.