Adaptation to a latitudinal thermal gradient within a widespread copepod species the contributions of genetic divergence and phenotypic plasticity

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Title: Adaptation to a latitudinal thermal gradient within a widespread copepod species: the contributions of genetic divergence and phenotypic plasticity

Running title: Adaptation to latitudinal gradients

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***Abstract*:**

Understanding how populations adapt to heterogeneous thermal regimes is essential for comprehending how latitudinal gradients in species diversification are formed, and how taxa will respond to ongoing climate change. Adaptation can occur by innate genetic factors, by phenotypic plasticity, or by a combination of both mechanisms. Yet, the relative contribution of such mechanisms to large-scale latitudinal gradients of thermal tolerance across conspecific populations remains unclear. We examine thermal performance in 11 populations of the intertidal copepod *Tigriopus californicus*, ranging from Baja California Sur (Mexico) to British Columbia (Canada). Common garden experiments show that survivorship to acute heat stress differs between populations (by up to 3.8°C in LD_{50} values), reflecting a strong genetic thermal adaptation. Using a split-brood experiment with two rearing temperatures, we also show that developmental phenotypic plasticity is beneficial to thermal tolerance (by up to 1.3°C), and that this effect differs across populations. Although genetic divergence in heat tolerance strongly correlates with latitude and temperature, differences in the plastic response do not. In the context of climate warming, our results confirm the general prediction that low-latitude populations are most susceptible to local extinction because genetic adaptation has placed physiological limits closer to current environmental maxima, but our results also contradict the prediction that phenotypic plasticity is constrained at lower latitudes.

Introduction

Latitudinal gradients in abiotic factors strongly determine species distributions. Of these factors, temperature is arguably the most important, as its effects are pervasive across all levels of biological organization, from biochemistry of molecular processes to physiology of the whole organism [1-3]. As such, it is expected that organisms adapt to latitudinal gradients by matching their physiological tolerances to the local thermal environment experienced along their range [4]. Understanding the mechanisms underlying the evolution of thermal tolerance is critical not only for explaining contemporary biogeographic patterning, but also for predicting how climate change and evolutionary adaptation will affect future species distributions [5,6].

Adaptation is generally defined as a genetically-based change caused by natural selection [7,8]. Comparative physiology studies involving populations of one species or congeneric species spanning a wide latitudinal gradient have most clearly identified two non-mutually exclusive mechanisms underlying thermal adaptation: innate genetic divergence and phenotypic plasticity [6]. Divergence of genomic regions involved in thermal tolerance can alter protein expression, structure, and performance in different environments, affecting individual fitness by natural selection and ultimately leading to fixation of advantageous mutations in populations. This clear link between mutation, gene, function and phenotype, has made studies of genetic divergence appealing and prominent in the adaptation literature [9]. Studies have shown that even single amino-acid substitutions in orthologous enzymes from taxa adapted to different temperatures may significantly alter the structure and function of proteins [10-12]. On the other hand, phenotypic plasticity allows the same genotype to adjust the phenotype when induced by environmental cues [13]. Although the genetic basis of plasticity remains debated [8,14], it is now clear that plasticity can be under genetic control, specific to certain functions, altered by natural selection [14-16], and hence provide an underappreciated mechanism for adaptation [17]. Common garden experiments have shown that the contribution of plastic responses to thermal tolerance can be equal to or even greater than that of innate genetic divergence [18]. A renewed interest in phenotypic plasticity has motivated studies showing that these two mechanisms may interact to impede or promote phenotypic evolution [15,19], even ecological speciation [20-22].

Two predictions can be made in regards to how these two mechanisms of adaptation will influence taxa spanning large-scale latitudinal gradients of temperature: innate genetic responses should correlate with mean environmental temperature [23], while phenotypic plasticity (or performance breath) should correlate with the amplitude of thermal variability [24] (following the climatic variability hypothesis [25]). Studies of porcelain crabs [26,27] and tropical lizards [28] have shown some support for these hypotheses. Those results imply that warm-adapted taxa from lower latitudes, particularly ectotherms, are most threatened by further increases in temperature because 1) their innate acute thermal limits (LD_{50} values) lie close to current environmental thermal maxima, and 2) they have only limited ability to modify heat-tolerance through plasticity [6].

We address these hypotheses using the marine copepod *Tigriopus californicus*, which is widely distributed over a large-scale (~35°) latitudinal gradient in North America, ranging from Baja California Sur to Alaska. Spatial restriction of this species to splash pools in the high intertidal and supralittoral zone, where tidal immersion is rare, has resulted in allopatric evolution [29]. Independently evolving populations along this latitudinal gradient experience several similar abiotic stressors, such as variation in salinity, oxygen and pH, as is characteristic of high tidal pools [30,31]. However, other abiotic stressors such as temperature show a pronounced clinal variation, both in temperature of the air and sea surface. Some populations experience temperatures up to 40° C [32], occasionally leading to pool desiccation and local extinctions [30]. Common garden experiments with populations spanning up to 17° of latitude have shown that survivorship to acute thermal limit decreases with increasing latitude, suggesting that copepod populations are genetically adapted to a latitudinal thermal gradient [32,33]. Transcriptome analysis has shown that expression of orthologous heat-shock proteins strongly differs between a warm- and a cold-adapted population raised in common garden [34], indicating that high inter-population differentiation might facilitate genetic adaptation, via both structural changes in proteins and changes in gene expression. An experiment rearing descendants of wild-caught females from 6 populations at two temperature regimes (cold and constant versus warm and variable) suggested that rearing regime (mean temperature or variability) causes a plastic response in thermal tolerance, and that plasticity seemed to be inversely correlated with latitude [32]. Yet, the potential contribution of phenotypic plasticity to adaptation to latitudinal thermal gradients remains unclear, as it requires split-brood experiments [13].

To assess the relative contribution of genetic divergence and phenotypic plasticity in the adaptation to a latitudinal temperature gradient by the copepod *T. californicus* we focus on 11 natural populations spanning 23.4° of latitude which have evolved under a wide range of temperature regimes. First, we test if genetic divergence contributes to population differences in heat tolerance, using common garden experiments that exclude trans-generational maternal effects, and measuring survivorship to acute heat-stress from 33°C to 40°C. Second, we test if developmental phenotypic plasticity contributes to heat-tolerance and whether it differs among populations by using a split-brood experiment with two rearing temperatures (constant 20 and 25°C). Finally, we examine correlation of both genetic and plastic adaptation with habitat temperature, providing insights on the relative importance of the two mechanisms potentially underlying thermal adaptation across this large-scale latitudinal gradient, and on population susceptibility to ongoing climate warming.

Materials and Methods

Population sampling and culturing

We extended the sampling from previous works [32,33] to encompass most of the species range by collecting a total of 11 populations (Fig. 1; Suppl. Table 1), from Baja California Sur in Mexico (27.18° latitude) to British Columbia in Canada (50.58° latitude). Stocks were maintained in 400 mL beakers of filtered seawater. Copepods were fed ground "Algae Wafers" (Kyorin Co., Himeji, Japan). Populations were split into additional beakers when they reached high densities and beakers were periodically mixed to prevent intrapopulation divergence due to stochastic genetic drift. Salinity was monitored and kept at a constant 32 ppt. Stock cultures were maintained at 20°C with a 12:12 L:D photoperiod for at least two generations before the split-brood experiment, to eliminate epigenetic and other maternal effects that can influence thermal tolerance [35].

Split-brood experiment and common garden

To account for intra-population genetic variation, ~200 gravid females from each stock population were randomly selected and pooled in petri dishes, which were kept for two days at 20°C. After this time, adult females were removed, and the descendent broods were randomly split into two different rearing temperatures: a control condition of 20°C considered to be benign across populations, and a treatment condition of 25°C that is expected to affect multiple life-history traits [36]. Contrary to a previous work addressing the effect of developmental phenotypic plasticity [32], temperature was kept constant in both control and treatment conditions, to avoid the known confounding effect of mean temperature and daily variability [36]. Nauplii were fed ad libitum, similar to stock cultures. Split-broods developing at control and treatment conditions reached maturity after four weeks, and were subsequently used for estimating thermal tolerance.

Survivorship to acute heat-stress

To measure thermal tolerance, we examined survivorship to acute heat-stress following the protocol developed by [33]. For each pair of control and treatment split-broods, ten mature animals were moved to 15 mL Falcon tubes with 10 mL of filtered seawater. After a resting period of one hour at their respective rearing temperature, the tubes were moved to a water bath at the target stress-temperature for one hour. Following the heat-stress, tubes were moved to 20°C for a one-hour period of recovery and then transferred to fresh medium. Survivorship was scored as the fraction of individuals surviving after three days.

Survivorship to acute heat-stress was assessed at one degree intervals from 33°C to 40°C, depending on the range of temperatures tolerated by each population (Suppl. Table 2). The assay was replicated six times for mid-range heat-stress temperatures, where we expected to see the greatest difference in survivorship between rearing conditions, and three times for upper and lower heat-stress temperatures, where survivorship of split-broods in alternative rearing conditions converged. Each heat-stress experiment included control and treatment split-broods, as well as populations from different latitudes, so that genetic differences were not confounded by possible temporal variations of the assay. This study includes 42 to 66 replicates per population, corresponding to a total of 6,180 adult copepods.

Contribution of genetic divergence

To test if genetic divergence contributes for population differences in heat tolerance, we compared survivorship of populations growing under common garden conditions, both at 20 and at 25°C. To describe the thermal performance curves for each population (one for each rearing condition), we first calculated mean and standard error for each heat-stress temperature and combined values across temperatures. We then produced a model of thermal tolerance for each population and rearing condition, by fitting a sigmoidal curve to all replicates of survivorship across all heat-stress temperatures. From this model we calculated the thermal tolerance as expressed by LD_{50} (i.e. temperature inducing 50% survivorship), thermal limit as expressed by LD_{10} (i.e. temperature inducing 10% survivorship), and the respective standard errors. Based on previous work with more limited sampling and fewer heat-stress temperatures, we expect southern populations to be more heat-tolerant than northern populations [32,33]. All statistical analyses were performed in R 2.15.1 (R Development Core Team, functions: glm, dose.LD50).

Contribution of phenotypic plasticity

To test if developmental phenotypic plasticity contributes to heat tolerance, we first tested whether survivorship of split-broods growing at different rearing conditions (20°C vs. 25°C) had significantly different survivorship to heat-stress, using a nonparametric Wilcoxon test on survivorship data at each heat-stress temperature (R function: wilcox.test). We then used LD_{50} values to plot reaction norms of heat tolerance for each population at the two different rearing conditions. If higher rearing temperature results in 'adaptive' phenotypic plasticity in heat tolerance (i.e. beneficial [8]), we expect LD_{50}s for a given population to be significantly different between rearing temperatures and the reaction norms to have a positive slope. We assessed significance using a two-sample t-test.

If populations have genetic variance in phenotypic plasticity, we expect that those slopes will differ among pairs of populations, possibly generating crossed reaction norms [13]. First, we test if heat tolerances of split-broods reared at alternative conditions differ among the 11 populations. We fit the survivorship to heat-stress in a general linear model where survivorship is explained by heat-stress temperature, rearing condition, population, and the interaction between population and rearing condition. We then use the heterogeneity of slopes test (ANOVA) to explicitly test for significance of the interaction term, i.e. if populations differ in their phenotypic plasticity. Second, we identify which pairs of population have crossed reaction norms. For each pair of populations, we statistically tested for crossed reaction norms by: 1) calculating a test statistic reflecting the difference between the differences in LD_{50}s between populations at each rearing condition (test statistic = (LD_{50} pop1 20°C – LD_{50} pop2 20°C) – (LD_{50} pop1 25°C – LD_{50} pop2 25°C)); calculating a SE for that statistic using the sum of the squares of the SEs ($SE = \sqrt{\Sigma SE_{i,j}^2}$, where i is pop and j is rearing temperature); and finally calculating a z-score to reflect significance (z-score = test statistic / SE). We used a Bonferroni correction for multiple testing.

To better understand if adaptive phenotypic plasticity is caused by increases of thermal tolerance (LD_{50}) or thermal limit (LD_{10}) at each population, for each split-brood pair we calculated ΔLD_{50} and ΔLD_{10} and associated SEs, as described above.

Latitudinal Gradients

To understand how genetic divergence and phenotypic plasticity contribute to large-scale gradients on thermal tolerance, we tested correlations between latitude and air temperature (alternative independent variables) and indexes reflecting the two evolutionary mechanisms (dependent variables: LD_{50} at the benign rearing temperature of 20°C and ΔLD_{50}, respectively). We assessed statistical significance by fitting a linear model relating the dependent and independent variables (R function: lm). Annual mean air temperature and annual temperature range were recovered from the BioClim database for each site [37], at 1 km^2 resolutions and averaged across 30 years (~1560 generations of copepods).

Potentially confounding factors

Because elevated rearing temperatures are known to cause lower developmental survivorship [36], the adult individuals measured for thermal tolerance might represent a non-random subset of the population, thus leading to an increase of estimated thermal tolerance that reflects selection favoring tolerant individuals, rather than phenotypic plasticity. We tested this by measuring survivorship during development at the two rearing temperatures. During the split-brood experiments, we transferred 10 nauplii (times 12 replicates) from each population into six-well plates and reared them at control and treatment temperatures, as explained above. After 14 days, we measured developmental survivorship and used a Wilcoxon test to assess significance between rearing temperatures. If there is an effect of selection during development, we expect a positive correlation between difference in developmental survivorship (a proxy for the strength of selection) and increase in thermal tolerance (ΔLD_{50}).

In addition to long-term developmental conditions discussed above, phenotypic plasticity may also result from short-term acclimation of adult individuals. We tested whether acclimation can result in similar changes in thermal tolerance observed after full development at different temperatures. For three populations from different latitudinal ranges for which we had abundant stock cultures (PAC, PES, BR), we randomly sampled 10 adults (times 6 replicates) into petri dishes, and kept them at 20 or 25°C for one day. After acclimation, we assessed survivorship to acute heat-stress at 36°C as explained above; this heat-stress temperature was previously identified as resulting in a strong increase in thermal tolerance across most populations. We used a Wilcoxon test corrected for multiple comparisons to assess differences on survivorship between control temperature (stable at 20°C), acclimation (1 day at 25°C), and development (4 weeks at 25°C).

Results

Contribution of genetic divergence

Our split-brood and common garden experiment allowed us to describe the thermal performance curve for each population at the two rearing conditions (Fig. 1). In general, thermal performance curves approximated a sigmoidal shape, starting with a plateau of maximum survivorship at lower temperatures characterized by narrower standard errors, followed by a steep decrease of survivorship characterized by wider standard errors, and ending when we observed no survivorship. Performance curves from northern populations generally shift to lower temperatures, reaching zero survivorship at heat-stress temperatures where southern populations can maintain 100% survivorship (e.g. PAC vs SRQ; Fig. 1). This pattern is consistent across rearing conditions (Suppl. Fig. 1). The sigmoidal model shows differences in thermal tolerance (expressed by LD_{50}), varying between 33.813 °C in RC and 37.638°C in SRQ (Suppl. Fig. 2A); thermal limit (expressed by LD_{10}) varies between 34.754 °C in RC and 39.203 °C in SRQ (Suppl. Fig. 2B).

Contribution of phenotypic plasticity

Comparisons of performance curves between split-broods that developed under different rearing conditions show significant differences at one to four heat-stress temperatures within each population (asterisks in Fig. 1). In each population, split-brood pairs exhibited performance curves with the same approximate shape (Fig. 1), and the sigmoidal model shows significant increases in LD_{50} and LD_{10} in every population (Suppl. Fig. 2) in a generally proportional way across populations (Suppl. Fig. 3). The degree of phenotypic plasticity among populations is reflected by increases of thermal tolerance, as expressed by ΔLD_{50}, and varies from 0.396 to 1.341°C (Suppl. Fig. 4). All reaction norms for heat tolerance (LD_{50}) have a positive slope (Fig. 2). The model shows that survivorship strongly depends on heat-stress temperature and rearing condition (*P*-values $<< 0.001$), and that in some populations the effect of rearing condition is significantly different from the average effect (e.g. HEC and BUF among others; see Suppl. Table 3 for *P*-values). The ANOVA showed that all four terms of the model, including the interaction between population and rearing condition, significantly contribute to explain the observed survivorship to heat stress (all *P*-values << 0.001). This variation of phenotypic plasticity among populations is also reflected in crossed reaction norms based on LD_{50}. Among all 55 pairwise comparisons, 25 pairs of populations have a z-score > 1.96 (corresponding to an α of 0.05 in a two-sided test). From these, 10 pairwise comparisons remain significant after correcting for multiple testing (z-score > 3.26; Suppl. Table 4), 6 of which involve HEC, 3 involve BUF, and 1 is PES x RC.

Latitudinal Gradients

Thermal tolerance, as expressed by LD_{50}, is strongly negatively correlated with latitude ($R^2 = 0.831$, *P*-value = 10^{-5}; Suppl. Fig. 5A). This is reflected by positive correlation with temperature ($R^2 = 0.8182$, *P*-value = $8x10^{-5}$; Fig. 3A).

In contrast, we find that the plasticity in thermal tolerance, as expressed by ΔLD_{50}, is neither significantly correlated with latitude ($R^2 = 0.0657$, *P*-value = 0.5; Suppl. Fig. 5B) nor with temperature ($R^2 = 0.01326$, *P*-value = 0.736; Fig. 3A).

Potentially confounding factors

We did not find any evidence for an effect of confounding factors on our results. Although survivorship during development is slightly lower at warmer rearing temperatures, differences are only significant for 3 out of the 11 populations (Wilcoxon pairwise comparison for BDM, HEC and RC: *P*-values < 0.05; Suppl. Fig. 6A). Most importantly, the observed increases in heat tolerance (ΔLD_{50}) are not positively correlated with the strength of selection during development, as expressed by difference in survivorship at the two rearing temperatures (in fact the correlation is negative, R^2= 0.31, *P*-value= 0.047; Suppl. Fig. 6B). We observed slight increases in survivorship to heat-stress after short-term acclimation, but survivorship of acclimated individuals is not significantly different from control individuals lacking acclimation (all *P*-values > 0.55). Survivorship for both acclimated and non-acclimated individuals is significantly lower than for individuals that developed at warmer rearing temperature (all *P*-values < 0.024; Suppl. Fig. 7).

Discussion

Genetic divergence leads to large differences in thermal tolerance

Previous common garden experiments have shown that populations of *T. californicus* have evolved genetic differences in their tolerance to acute heat stress [32,33]. By extending this approach to 11 populations distributed along wider latitudes (23.4°, ranging from temperate to tropical environments) we confirm that these earlier findings are generalizable across the species range sampled here. The thermal performance curves (Fig. 1, Suppl. Fig. 1) show that, across all heat-stress temperatures, northern populations have a lower survivorship than southern populations. Differences in survivorship to heat-stress are most remarkable at 35°C, a temperature that frequently occurs in tide pools from California [32] and that does not lead to mortality in southern populations while being partially or fully lethal in northern populations. Notably, performance curves from some of the northernmost populations (RC, PAC, and BDM) plateau below 100% survivorship at lower heat-stress temperatures irrespective of the rearing conditions, showing that even relatively mild heat-stress (33 and 34°C) will cause some mortality at those populations but not in intermediate or lower latitude ones. Thermal tolerance and thermal limit, as estimated by LD_{50} and LD_{10}, are in agreement, with inter-population differences of up to 3.825 and 4.449°C respectively (Suppl. Fig. 2), showing significant genetic adaptation across conspecific populations.

Genetic adaptation between populations of the same species is often opposed by gene flow. In *T. californicus*, there is abundant evidence that gene flow is extremely restricted over the geographic range of the species [38-40]. Polymorphism studies across the transcriptome have shown that shared mutations quickly become fixed during population divergence [41]; e.g. between the closely related SD and BR populations, only 0.7% of the mutations are shared and 72.2% are fixed, with the remaining being polymorphic within each population. While such fixation at early stages of population divergence is likely driven by neutral evolution, this process also affects genes underlying thermal tolerance, such as heat-shock proteins, which contain amino-acid substitutions between the thermal tolerant population of SD and the relatively thermal sensitive population of SCN [34]. The populations studied here are distributed over a wider geographic and ecological range than previous studies and likely diverged in isolation over thousands or millions of years [42]. In this context, a combination of population demography, long divergence times and selection for alternative thermal regimes has apparently resulted in the accumulation of adaptive genetic differences among *T. californicus* populations and may explain the large difference in innate thermal tolerance observed here.

Adaptive phenotypic plasticity differs among populations

Similar to studies on other species, most investigations of the evolution of thermal tolerance in *Tigriopus californicus* have aimed to exclude phenotypic plasticity in order to concentrate on genetic adaptation. Here, we show that 1) performance curves of split-broods reared at different temperatures show significant differences of survivorship to acute heat stress (Fig. 1) and 2) reaction norms from all populations are positively sloped (Fig. 2), clearly supporting a significant beneficial contribution of phenotypic plasticity to thermal tolerance. Phenotypic plasticity in thermal tolerance is characterized both by an increase in thermal tolerance (LD_{50}) and in thermal limit (LD_{10}), generally leading to a shift of the whole performance curve to higher temperatures (Fig. 1, Suppl. Fig. 3). These increases in thermal tolerance were not confounded by differential survivorship due to rearing condition (Suppl. Fig. 6), indicating that differences in phenotype are indeed caused by developmental phenotypic plasticity rather than selective mortality during development. Moreover, individuals reared at higher temperature show a significantly higher survivorship to acute heat stress compared to individuals acclimated to the same temperature for 1 day (Suppl. Fig. 7), suggesting that developmental phenotypic plasticity can result in much larger effects than short-term plasticity. Yet, changes in thermal tolerance due to adaptive plasticity are only up to 1.34 °C, much smaller than the 3.83 °C inter-population difference due to genetic adaptation (Suppl. Fig. 2). Reviews on thermal adaptation to climate change suggest that plastic factors seem to be more to important than genetic factors [5]. Yet, the few studies successfully separating both factors tend to be restricted to fruit flies and daphnia [18,43]. Our results contradict this, suggesting that generalizations are still premature until a larger variety of taxa are studied.

Interestingly, our results show that the magnitude of phenotypic plasticity differs among populations, resulting in a significant interaction between genotype and environment (i.e. between population and rearing temperature) and in crossed reaction norms (Fig. 2). This pattern is indicative of some genetic variation in plasticity [13,44], suggesting that populations of *T. californicus* differ genetically in the plastic response to heat stress. Although the genetic basis of phenotypic plasticity remains largely unknown, genetic (heritable) variation is the minimal requirement for the maintenance of plasticity by natural selection [8,13,45]. When alterations in the environment are predictable, such as during global warming, the ability to flexibly and rapidly respond to a new environment will be associated with increased fitness, allowing plasticity to evolve via Darwinian evolution. Recent studies in cichlid fishes have shown that plasticity in feeding morphology is genetically determined and can evolve adaptively via genetic assimilation [16], leading to ecological diversification and eventually to species formation [22]. Our finding of genetic variance in plasticity for thermal tolerance, which has direct consequences to individual physiology and fitness, suggests that phenotypic plasticity may in fact evolve via natural selection and contribute to adaptation to heat stress.

Latitudinal gradients in thermal tolerance correlate with innate genetic differences, but not with developmental plasticity

Heritable clinal patterns for temperature stress resistance have been described in a number of terrestrial invertebrates. Such macro-ecological patterns are hypothesized to reflect both genetic adaptation to higher temperatures characteristic of lower latitude, and/or plastic adaptation to broader temperature ranges experienced at higher latitudes (termed as the climatic variability hypothesis; [25]). By successfully separating genetic and plastic adaptation to heat stress in widespread populations of marine copepods, we test if both mechanisms contribute to large latitudinal gradients of thermal tolerance.

In *T. californicus*, we find a strong and significant correlation of thermal tolerance, as reflected by LD_{50}, both with mean air temperature (Fig. 3A) and with latitude (Suppl. Fig. 5A). These strong correlations between innate thermal tolerance and habitat measurements strongly suggest that mean temperatures experienced by local populations are a major driver of the large latitudinal gradients of thermal tolerance observed in this species. Studies in other species have shown similar correlation of innate, or environmentally independent, thermal tolerance with latitude (e.g. porcelain crabs [26], marine mollusks [46], amphibians [47] and fruit flies [48]), suggesting that genetic adaptation strongly contributes to large-scale latitudinal gradients observed across species. An emerging implication of latitudinal gradients of innate thermal tolerance across terrestrial insects [49], also supported by our results in this marine copepod, is that warm-adapted populations from lower latitudes are characterized by thermal limits that are frequently experienced in their local environments, suggesting that southernmost populations might be close to their innate adaptive capacity.

The two critical assumptions of the climate variability hypothesis are that there is a gradient between latitude and thermal variability, and that there is a match between this cline and thermal physiological breath of a taxon [25]. Although there is a clear correlation between latitude and temperature variability throughout the range of *T. californicus* (Supp. Fig. 8), we do not find a correlation with performance breadth, as reflected by ΔLD_{50} (Fig. 3B), suggesting that phenotypic plasticity does not contribute to latitudinal gradients of thermal tolerance in this species. Our result contrasts with an earlier study using 6 populations from 3 similar environments [32], suggesting that broader sampling, a split brood experimental design, parallel rearing conditions and the careful exclusion of maternal effects are required for appropriate tests of the contribution of phenotypic plasticity in adaptation to large scale thermal gradients. Other factors that do not vary with latitude might be more relevant to explain the observed variation in phenotypic plasticity. The level of microclimate variability, such as the level of sun exposure of tide pools or pool size, varies strongly throughout the species range and can cause strong variation in temperature at finer spatial scales, which would not be recognized in the macro climatic variables used here. Other studies where genetic and plastic factors could be separated also did not show a correlation between latitude and thermal breath (e.g. in amphibians [50] and in fruit flies [51]), suggesting that the climatic variability hypothesis might not explain the evolution of latitudinal gradients in thermal tolerance in all species and that its generalization to explain such a strong macro-ecological pattern is premature.

Concerning current climate warming, species from lower latitudes are generally considered to be the biggest ‘losers’ of adaptive capacity [6]. Our results from *Tigriopus californicus* suggest that this generalization is only partially true. Although we confirm that adaptive genetic capacity of southern populations might be limited because thermal limits lie near current thermal maxima, phenotypic plasticity is not more restricted, providing some, yet limited, capacity to accommodate increases in heat-tolerance.

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Figure legends

Figure 1. Thermal tolerance along the distribution of *Tigriopus californicus*. Sampling localities (right map) span a latitudinal gradient from 27.18°N in the state of Baja California South, Mexico, to 50.58°N in the state of Columbia, Canada. Survivorship to heat stress was tested in siblings reared at a control optimal temperature (20°C; black) and a treatment sub-optimal temperature (25°C; grey). Performance curves connect mean survivorship ± SE to 1-hour acute heat stress, between 33 to 40 °C. Significant differences in survivorship between control and treatment are demarked by * for $P < 0.05$, and ** for $P < 0.01$ (Mann-Whitney *U*-test).

Figure 2. Reaction norms of thermal tolerance, after a split-brood experiment and two rearing temperatures (20 and 25 °C). Thermal tolerance is summarized by LD_{50}, which reflects the temperature causing 50% mortality; bars reflect standard errors, which are affected by technical replication and intra-population variability.

Figure 3. Relative contribution of genetic adaptation and phenotypic plasticity to large-scale latitudinal gradient of air temperature in *Tigriopus californicus*. A. Genetic adaptation as reflected by thermal tolerance (LD_{50}) in a common garden at a benign rearing temperature. B. Developmental phenotypic plasticity as reflected by increase of thermal tolerance (ΔLD_{50}) in split broods reared at different temperatures.

Supplementary Figure 1. Gradient of thermal tolerance in populations of *Tigriopus californicus* at the two rearing conditions. Populations are colored according to latitude, from the thermal sensitive populations from northern latitudes (blue tones), to the thermal tolerant populations from southern latitudes (red tones).

Supplementary Figure 2. Thermal tolerance to heat stress at individual populations. **A.** Thermal tolerance estimated as the temperature causing 50% survivorship (LD_{50}). **B.** Thermal limit estimated as the temperature causing 10% survivorship (LD_{10}). Stars indicate significant differences between split-broods reared at different temperatures ($\alpha = 0.05$).

Supplementary Figure 3. Effect phenotypic plasticity on thermal tolerance (LD_{50} ± SE) and lethal temperature (LD_{10} ± SE) for every population.

Supplementary Figure 4. Increase in thermal tolerance is summarized by ΔLD_{50} ± SE; populations are ordered from lower to higher latitude in the grey shade corresponding to the respective reaction norm (Fig. 2).

Supplementary Figure 5. Relative contribution of genetic adaptation and phenotypic plasticity to large-scale latitudinal gradient in *Tigriopus californicus*.

Supplementary Figure 6. Effect of mortality during development on increase on thermal tolerance. **A.** Survivorship at 14 days during development at 20 and 25 °C. Significant differences are demarked with * ($P < 0.05$). **B.** Correlation between difference of survivorship during development and increase on thermal tolerance reflected by ΔLD_{50}. A linear regression reveals a negative non-significant relation between survivorship during development and increase in thermal tolerance.

Supplementary Figure 7. Effect of acclimation on survivorship to heat-stress. Bars reflect mean survivorship (± SE) to acute heat stress of 36°C for a southern, a central and a northern population. Animals that developed at 20°C and were acclimated to 25°C for 24 hours did not significantly increase survivorship to heat stress relative to non-acclimated individuals, while all animals that developed at 25°C did (* for $P < 0.05$, and ** for $P < 0.01$; Mann-Whitney *U*-test).

Supplementary Figure 8. Correlation between latitude and range of air temperature (A) and mean air temperature (B) across sampled populations.

Supplementary Table 1. Sampling localities of *Tigriopus californicus*.

Supplementary Table 2. Number of replicates and mean survivorship to heat stress in split-broods from control (C) and treatment (T) conditions.

Supplementary Table 3. Coefficients of all terms (and categories) included in the model explaining survivorship to heat-stress.

Supplementary Table 4. Significance values (z-scores) for crossed reaction norms between every population pair.

References:

1. Hochachka, P. W. & Somero, G. N. 2014 *Biochemical Adaptation*. Princeton University Press. (doi:10.2307/j.ctt7zv9d4)

2. Somero, G. N. 1997 *Temperature Relationships: From Molecules to Biogeography*. Hoboken, NJ, USA: John Wiley & Sons, Inc. (doi:10.1002/cphy.cp130219)

3. Schmidt-Nielsen, K. 1997 *Animal physiology: adaptation and environment*. Cambridge University Press.

4. Gaston, K. J. & Spicer, J. I. 1998 Do upper thermal tolerances differ in geographically separated populations of the beachflea *Orchestia gammarellus* (Crustacea: Amphipoda)? *Journal of experimental marine biology and ecology*

5. Hoffmann, A. A. & Sgrò, C. M. 2011 Climate change and evolutionary adaptation. *Nature* **470**, 479–485. (doi:10.1038/nature09670)

6. Somero, G. N. 2010 The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and ‘losers’. *Journal of Experimental Biology* **213**, 912–920. (doi:10.1242/jeb.037473)

7. Stearns, S. C. 1986 Natural Selection and Fitness, Adaptation and Constraint. In *Patterns and Processes in the History of Life: Report of the Dahlem Workshop on Patterns and Processes in the History of Life Berlin 1985, June 16–21* (eds D. M. Raup & D. Jablonski), pp. 23–44. Berlin, Heidelberg: Springer Berlin Heidelberg. (doi:10.1007/978-3-642-70831-2_3)

8. Gotthard, K. & Nylin, S. 1995 Adaptive Plasticity and Plasticity as an Adaptation: A Selective Review of Plasticity in Animal Morphology and Life History. *Oikos* **74**, 3–17. (doi:10.2307/3545669?ref=search-gateway:89e1fd941716845ac8012b430ec94dc3)

9. Manceau, M., Domingues, V. S., Linnen, C. R., Rosenblum, E. B. & Hoekstra, H. E. 2010 Convergence in pigmentation at multiple levels: mutations, genes and function. *Philos T R Soc B* **365**, 2439–2450. (doi:10.1098/rstb.2010.0104)

10. Johns, G. C. & Somero, G. N. 2004 Evolutionary convergence in adaptation of proteins to temperature: A4-lactate dehydrogenases of Pacific damselfishes (*Chromis* spp.). *Mol. Biol. Evol.* **21**, 314–320. (doi:10.1093/molbev/msh021)

11. Fields, P. A., Rudomin, E. L. & Somero, G. N. 2006 Temperature sensitivities of cytosolic malate dehydrogenases from native and invasive species of marine mussels (genus *Mytilus*): sequence-function linkages and correlations with biogeographic distribution. *J. Exp. Biol.* **209**, 656–667. (doi:10.1242/jeb.02036)

12. Dong, Y. & Somero, G. N. 2009 Temperature adaptation of cytosolic malate dehydrogenases of limpets (genus *Lottia*): differences in stability and function due to minor changes in sequence correlate with biogeographic and vertical

distributions. *J. Exp. Biol.* **212**, 169–177. (doi:10.1242/jeb.024505)

13. Via, S. 1993 Adaptive phenotypic plasticity: target or by-product of selection in a variable environment. *Am Nat* **142**, 352–365.

14. Via, S., Gomulkiewcz, R., de Jong, G., Scheiner, S. M., Schlichting, C. D. & van Tienderen, P. H. 1995 Adaptive Phenotypic Plasticity - Consensus and Controversy. *Trends in Ecology & Evolution* **10**, 212–217.

15. 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 Ecology* **21**, 394–407. (doi:10.1111/j.1365-2435.2007.01283.x)

16. Parsons, K. J., Concannon, M., Navon, D., Wang, J., Ea, I., Groveas, K., Campbell, C. & Albertson, R. C. 2016 Foraging environment determines the genetic architecture and evolutionary potential of trophic morphology in cichlid fishes. *Molecular Ecology* **25**, 6012–6023. (doi:10.1111/mec.13801)

17. West-Eberhard, M. J. 2005 Developmental plasticity and the origin of species differences. *Proc. Natl. Acad. Sci. U.S.A.* **102 Suppl 1**, 6543–6549. (doi:10.1073/pnas.0501844102)

18. Hoffmann, A. A., Shirriffs, J. & Scott, M. 2005 Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Funct Ecology* **19**, 222–227. (doi:10.1111/j.1365-2435.2005.00959.x)

19. Pigliucci, M. 2001 *Phenotypic plasticity: beyond nature and nurture*. JHU Press.

20. Pfennig, W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D. & Moczek, A. P. 2010 Phenotypic plasticity's impacts on diversification and speciation. *TREE* **25**, 459–467. (doi:10.1016/j.tree.2010.05.006)

21. Thibert-Plante, X. & Hendry, A. P. 2010 The consequences of phenotypic plasticity for ecological speciation. *J Evolution Biol* **24**, 326–342. (doi:10.1111/j.1420-9101.2010.02169.x)

22. Schneider, R. F. & Meyer, A. 2017 How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. *Molecular Ecology* **26**, 330–350. (doi:10.1111/mec.13880)

23. Parmesan, C., Gaines, S., Gonzalez, L. & Kaufman, D. M. 2005 *Empirical perspectives on species' borders: environmental change as challenge and opportunity*. Oikos. (doi:10.1111/j.0030-1299.2005.13150.x)

24. Angilletta, M. J. 2009 *Thermal adaptation: a theoretical and empirical analysis*. New York: Oxford University Press Inc.

25. Stevens, G. C. 1989 The latitudinal gradient in geographical range: how so many species coexist in the tropics. *The American Naturalist*

(doi:10.1086/284913)

26. Stillman, J. H. & Somero, G. N. 2000 A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiol. Biochem. Zool.* **73**, 200–208. (doi:10.1086/316738)

27. Stillman, J. H. 2002 Causes and Consequences of Thermal Tolerance Limits in Rocky Intertidal Porcelain Crabs, Genus *Petrolisthes*. *Integrative and Comparative Biology* **42**, 790–796. (doi:10.2307/3885008?ref=search-gateway:dbe3a0aa2864b048493cd7a1d642b746)

28. Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Alvarez Pérez, H. J. & Garland, T. 2009 Why tropical forest lizards are vulnerable to climate warming. *Proc Biol Sci* **276**, 1939–1948. (doi:10.1098/rspb.2008.1957)

29. Burton, R. S. 1998 Intraspecific phylogeography across the Point Conception biogeographic boundary. *Evolution* **52**, 734–745.

30. Altermatt, F., Bieger, A. & Morgan, S. G. 2012 Habitat characteristics and metapopulation dynamics of the copepod *Tigriopus californicus*. *Mar. Ecol. Prog. Ser.* **468**, 85–93. (doi:10.3354/meps09994)

31. Vittor, B. A. 1971 Effects of the environment on fitness-related life history characters in *Tigriopus californicus*.

32. Kelly, M. W., Sanford, E. & Grosberg, R. K. 2011 Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proc. R. Soc. Lond. Series B* **279**, 349–356. (doi:10.1098/rspb.2011.0542)

33. Willett, C. S. 2010 Potential fitness trade-offs for thermal tolerance in the intertidal copepod *Tigriopus californicus*. *Evolution* **64**, 2521–2534. (doi:10.1111/j.1558-5646.2010.01008.x)

34. Schoville, S. D., Barreto, F. S., Moy, G. W., Wolff, A. & Burton, R. S. 2012 Investigating the molecular basis of local adaptation to thermal stress: population differences in gene expression across the transcriptome of the copepod *Tigriopus californicus*. *BMC Evol Biol* **12**, 170. (doi:10.1186/1471-2148-12-170)

35. Zizzari, Z. V. & Ellers, J. 2014 Rapid shift in thermal resistance between generations through maternal heat exposure. *Oikos* **123**, 1365–1370. (doi:10.1111/oik.01496)

36. Hong, B. C. & Shurin, J. B. 2015 Latitudinal variation in the response of tidepool copepods to mean and daily range in temperature. *Ecology* **96**, 2348–2359.

37. Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. & Jarvis, A. 2005 Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**, 1965–1978.

38. Burton, R. S. & Feldman, M. W. 1981 Population Genetics of *Tigriopus californicus*. II. Differentiation Among Neighboring Populations. *Evolution* **35**, 1192–1205. (doi:10.2307/2408132?ref=search-gateway:cb13ac8e8c475006b0b95c7b55cc95c9)

39. Burton, R. & Lee, B. 1994 Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. *Proceedings of the National Academy of Sciences* **91**, 5197.

40. Burton, R. S. 1997 Genetic evidence for long term persistence of marine invertebrate populations in an ephemeral environment. *Evolution* **51**, 993–998.

41. Pereira, R. J., Barreto, F. S., Pierce, N. T., Carneiro, M. & Burton, R. S. 2016 Transcriptome-wide patterns of divergence during allopatric evolution. *Molecular Ecology* **25**, 1478–1493. (doi:10.1111/mec.13579)

42. Peterson, D. L., Kubow, K. B., Connolly, M. J., Kaplan, L. R., Wetkowski, M. M., Leong, W., Phillips, B. C. & Edmands, S. 2013 Reproductive and phylogenetic divergence of tidepool copepod populations across a narrow geographical boundary in Baja California. *J Biogeography* **40**, 1664–1675. (doi:10.1111/jbi.12107)

43. Yampolsky, L. Y., Schaer, T. M. M. & Ebert, D. 2014 Adaptive phenotypic plasticity and local adaptation for temperature tolerance in freshwater zooplankton. *Proc Biol Sci* **281**. (doi:10.1098/rspb.2013.2744)

44. Via, S. 1987 Genetic Constraints on the Evolution of Phenotypic Plasticity. In *Genetic Constraints on Adaptive Evolution* (ed V. Loeschcke), pp. 47–71. Berlin, Heidelberg: Springer Berlin Heidelberg. (doi:10.1007/978-3-642-72770-2_4)

45. Gomulkiewicz, R. & Kirkpatrick, M. 1992 Quantitative genetics and the evolution of reaction norms. *Evolution* **46**, 390–411.

46. Kuo, E. & Sanford, E. 2009 Geographic variation in the upper thermal limits of an intertidal snail: implications for climate envelope models. *Mar. Ecol. Prog. Ser.* **388**, 137–146. (doi:10.3354/meps08102)

47. Snyder, G. K. & Weathers, W. W. 1975 Temperature Adaptations in Amphibians. *The American Naturalist* **109**, 93–101. (doi:10.2307/2459639?ref=search-gateway:a06be88779d8c67c0d6d6c744ac34c3c)

48. Hoffmann, A. A., Anderson, A. & Hallas, R. 2002 Opposing clines for high and low temperature resistance in Drosophila melanogaster. *Ecol Letters* **5**, 614–618. (doi:10.1046/j.1461-0248.2002.00367.x)

49. Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C. & Martin, P. R. 2008 Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*

105, 6668–6672. (doi:10.1073/pnas.0709472105)

50. Brattstrom, B. H. 1968 Thermal acclimation in anuran amphibians as a function of latitude and altitude. *Comp. Biochem. Physiol.* **24**, 93–111.

51. Overgaard, J., Kristensen, T. N., Mitchell, K. A. & Hoffmann, A. A. 2011 Thermal Tolerance in Widespread and Tropical Drosophila Species: Does Phenotypic Plasticity Increase with Latitude? *Am Nat* **178**, S80–S96. (doi:10.1086/661780)

Data accessibility statement:

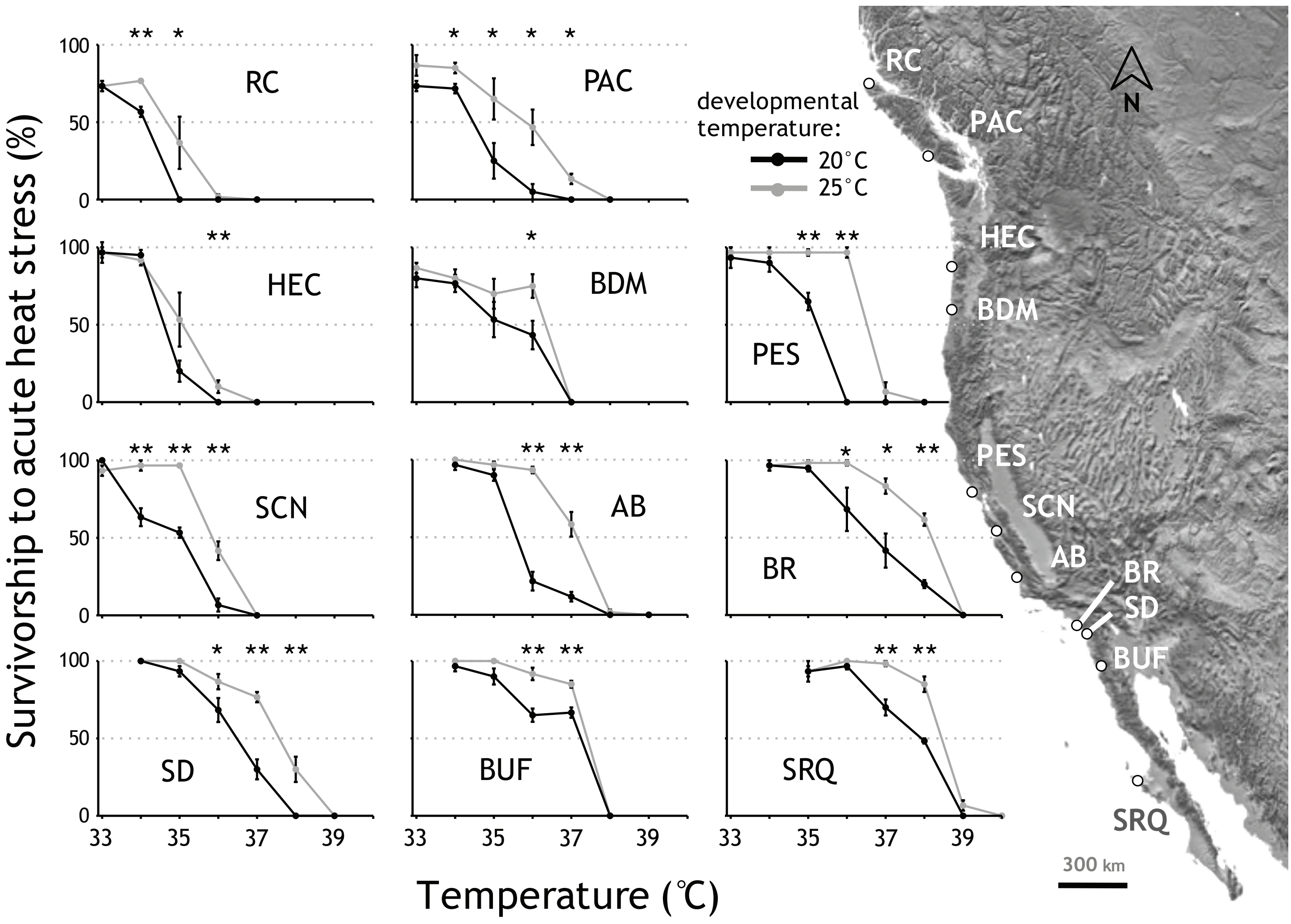
Estimates of thermal tolerance and GPS coordinates for collection sites are included as Supplementary Tables. Raw data on survivorship to acute heat stress and summary statistics of heat tolerance are archived in dryad repository (doi: 10.5061/dryad.bp76g).

Competing interests statement:

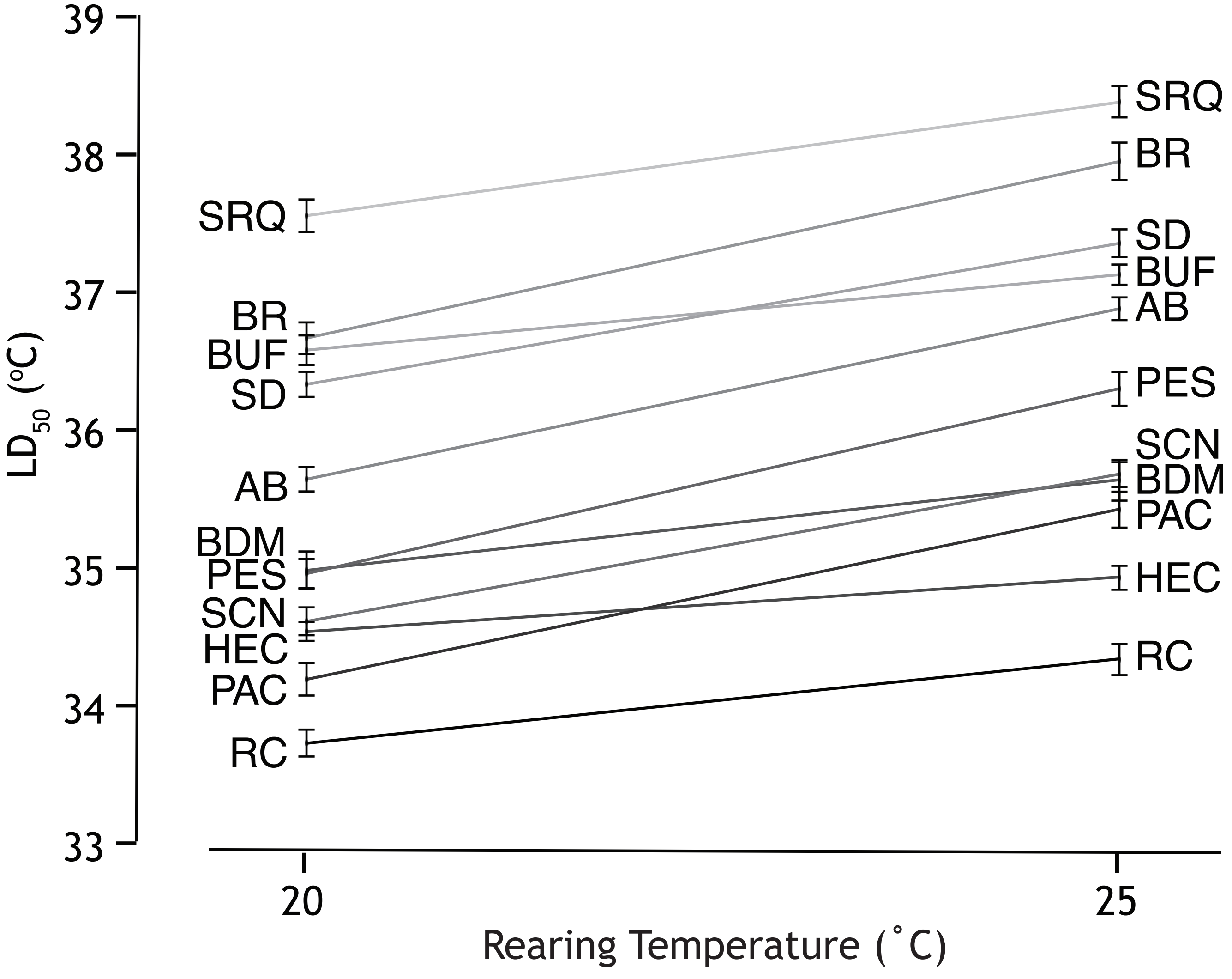
The authors have no competing interests.

Authors' contributions statement:

RJP conceived the project, collected the samples and developed the experimental design. MCS performed the experiments. RJP and MCS analyzed the data and wrote the first version of the manuscript. RSB contributed with laboratory resources, interpreting the data and preparing the final version of the manuscript.



A. Thermal Performance Curves



39
38
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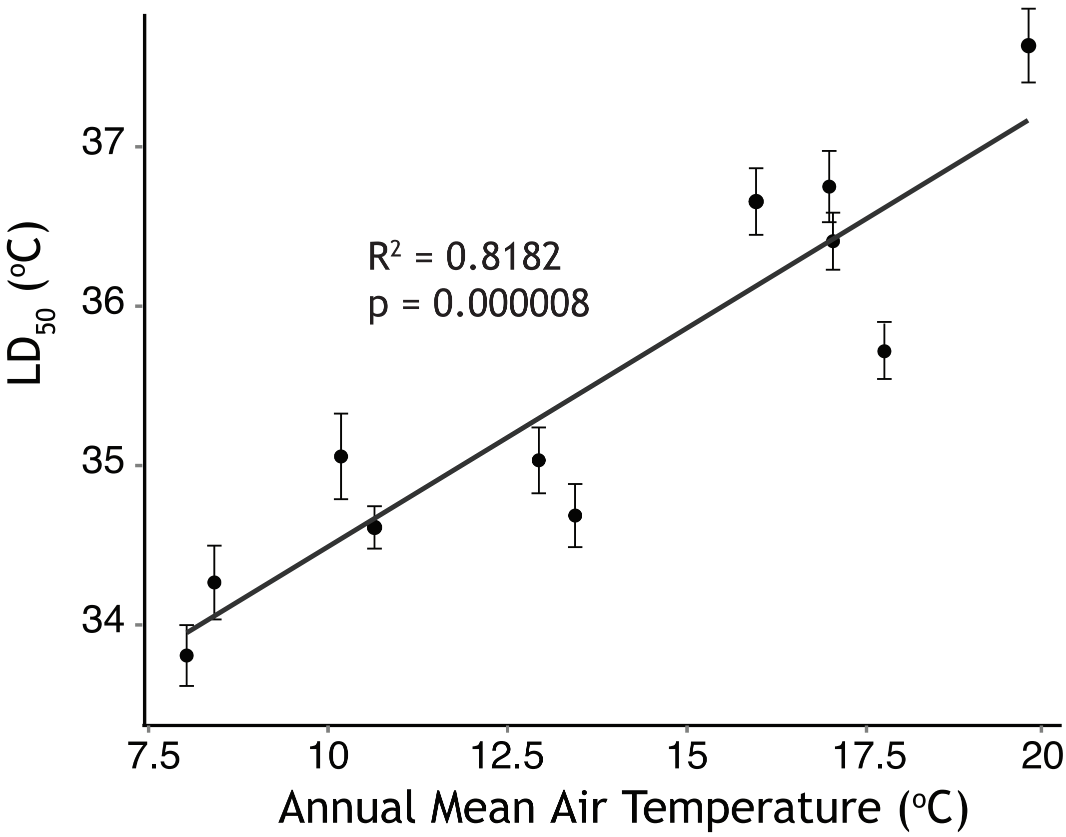
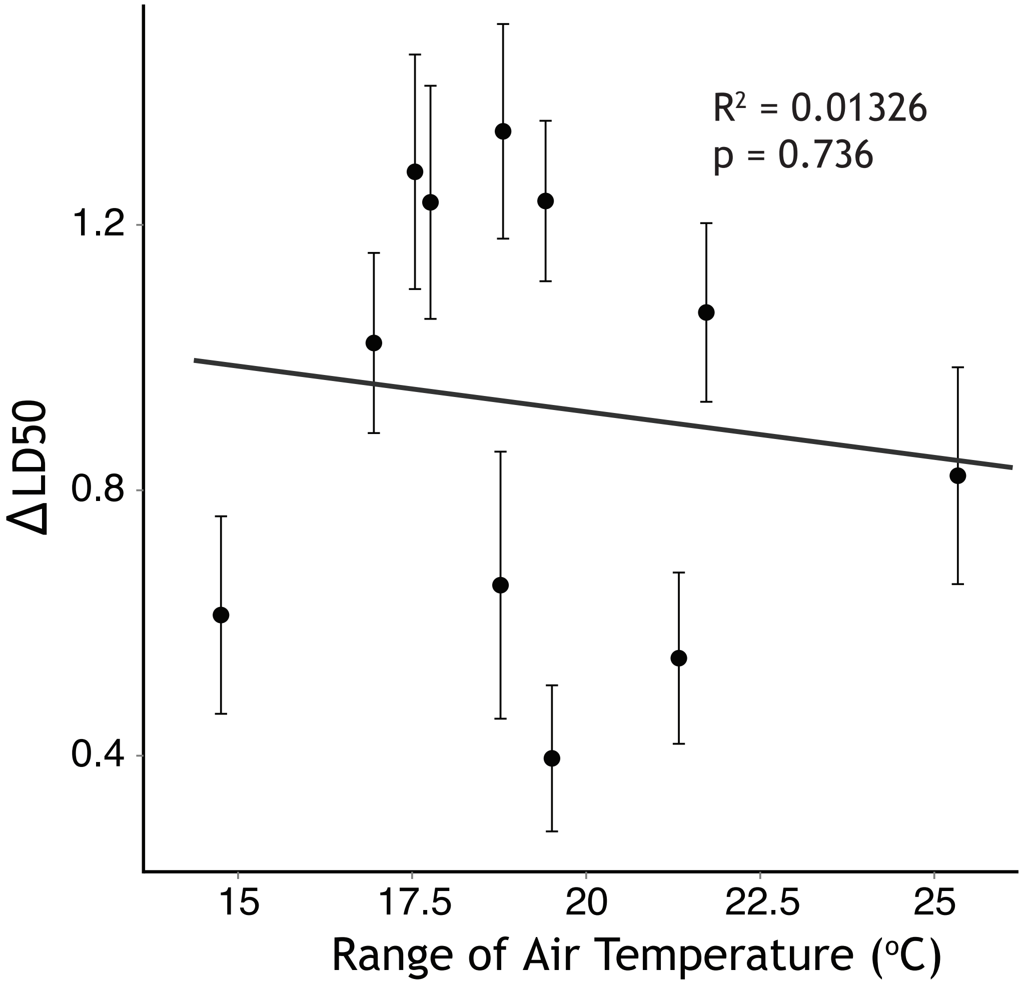
LD$_{50}$ (ºC)
20
25
Rearing Temperature (˚C)
SRQ
BR
BUF
SD
AB
BDM
PES
SCN
HEC
PAC
RC
SRQ
BR
SD
BUF
AB
PES
SCN
BDM
PAC
HEC
RC

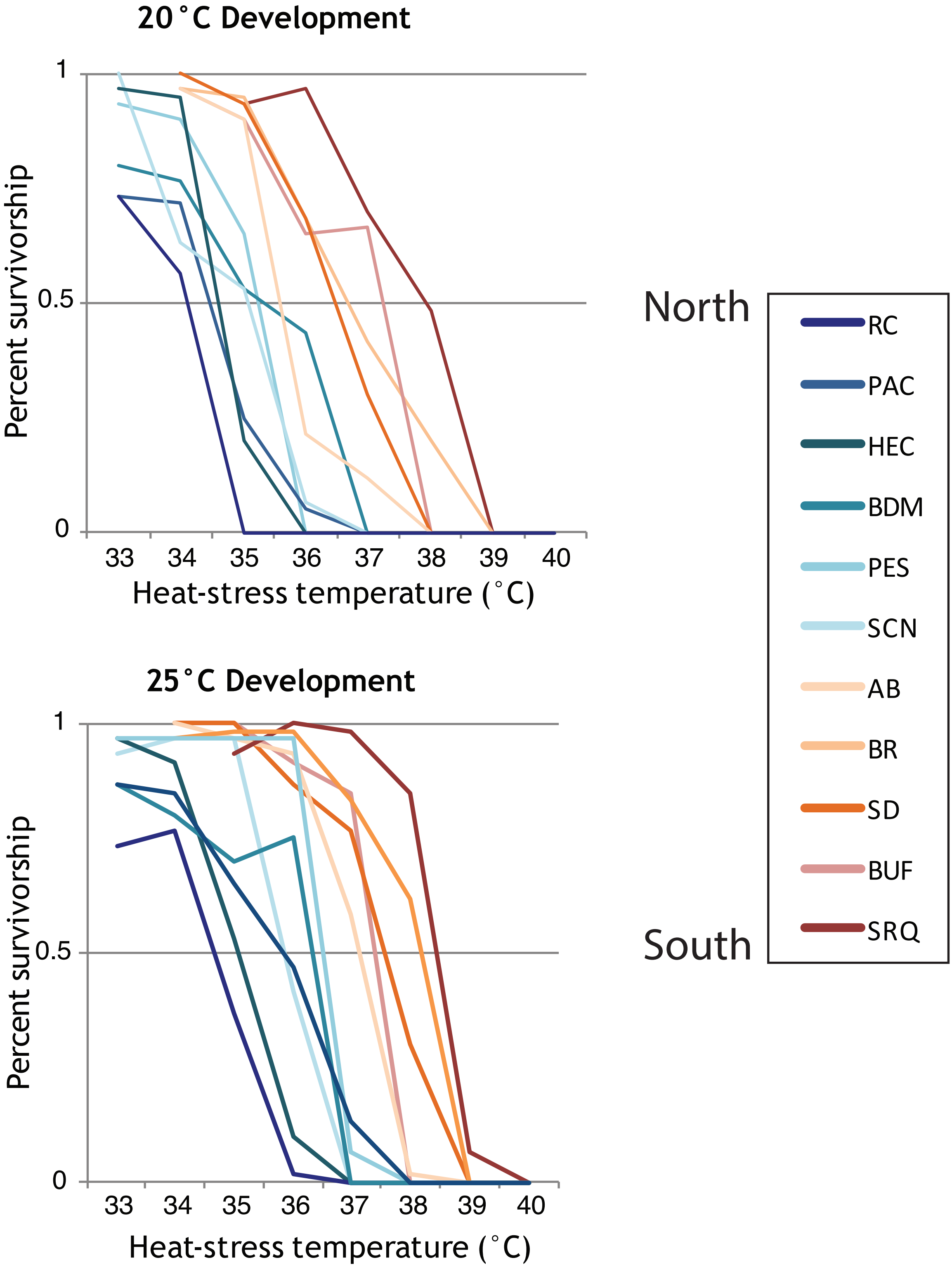


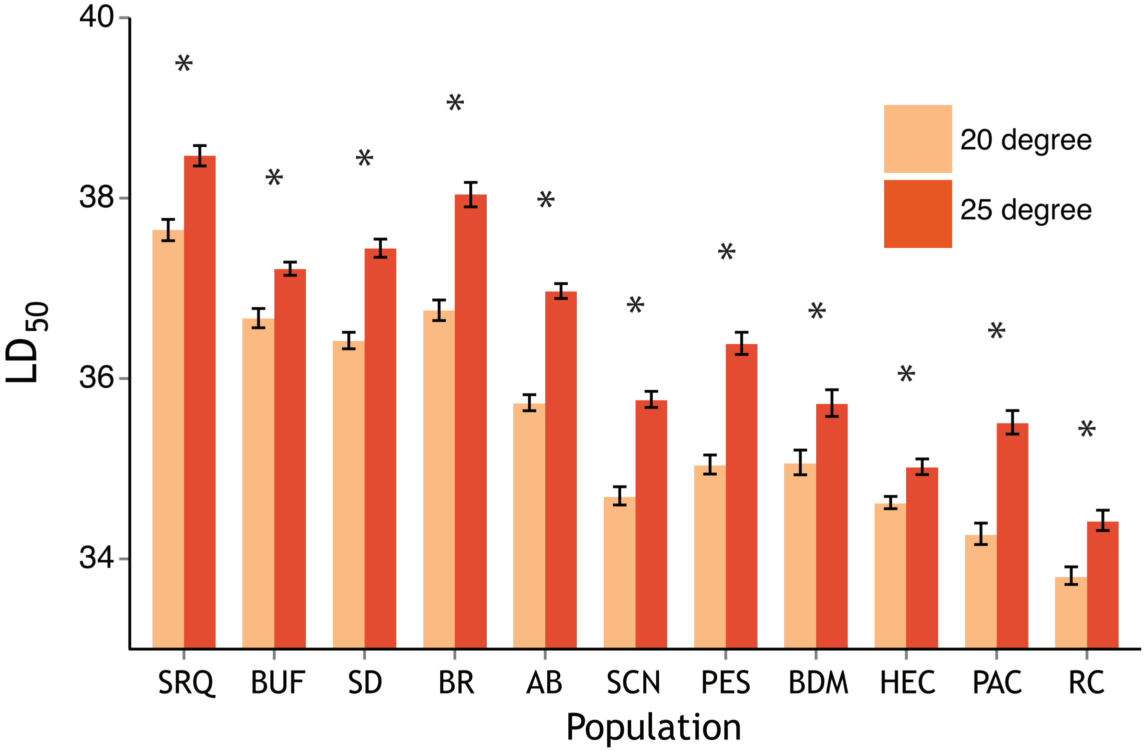
A. LD50 and Mean Air Temperature



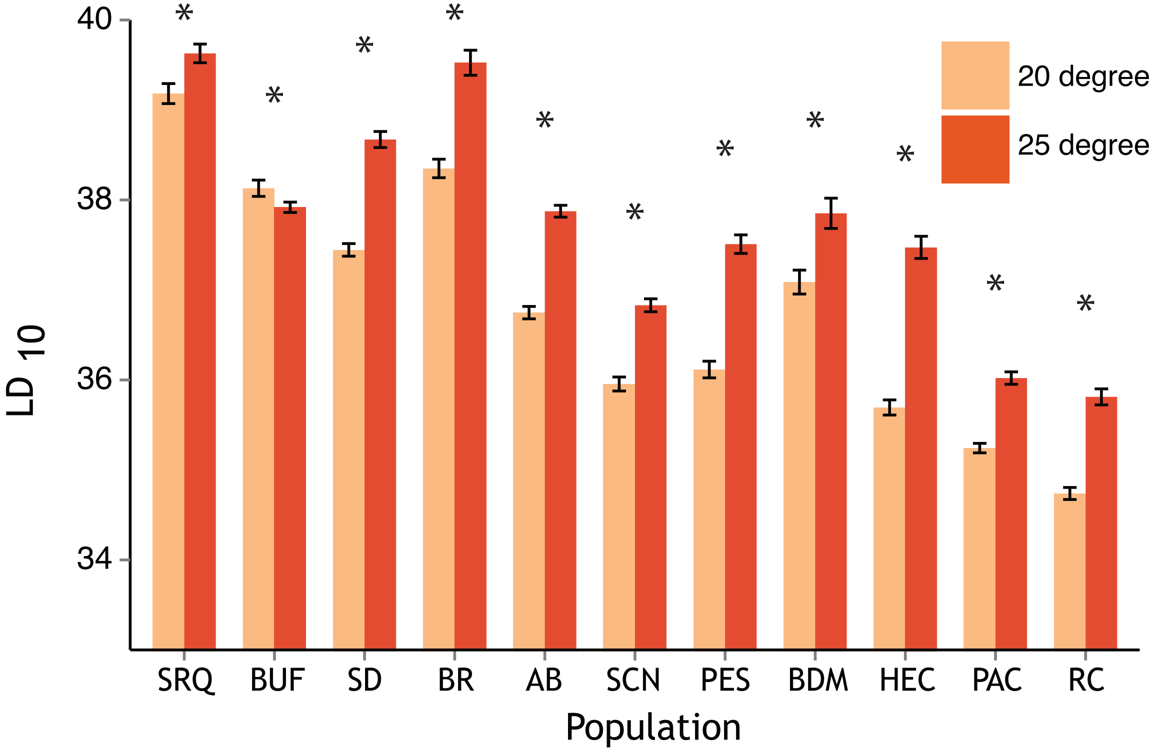
B. ΔLD50 and Range in Air Temperature

A. All Thermal Performance Curves



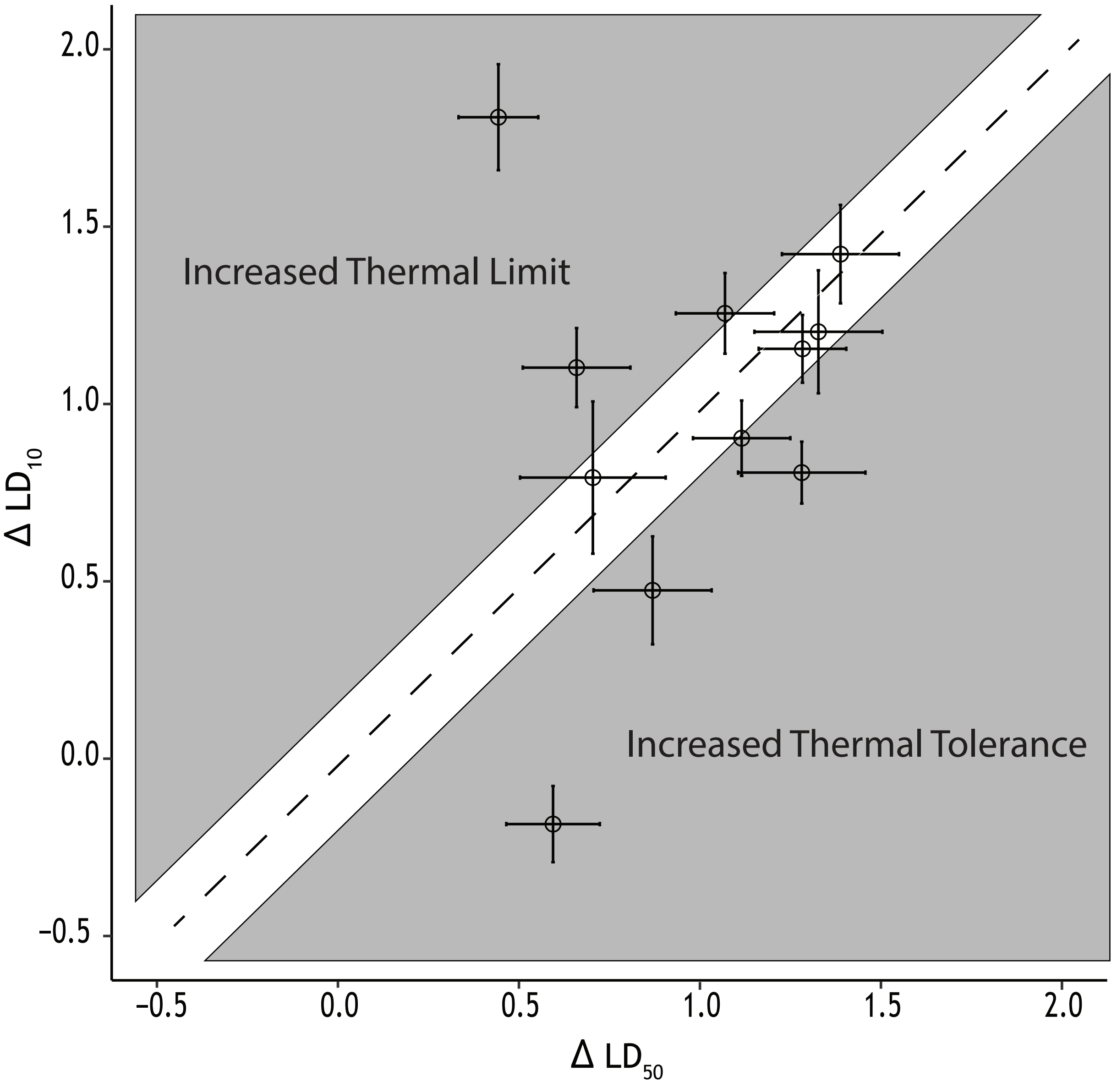


A. Thermal Tolerance by population

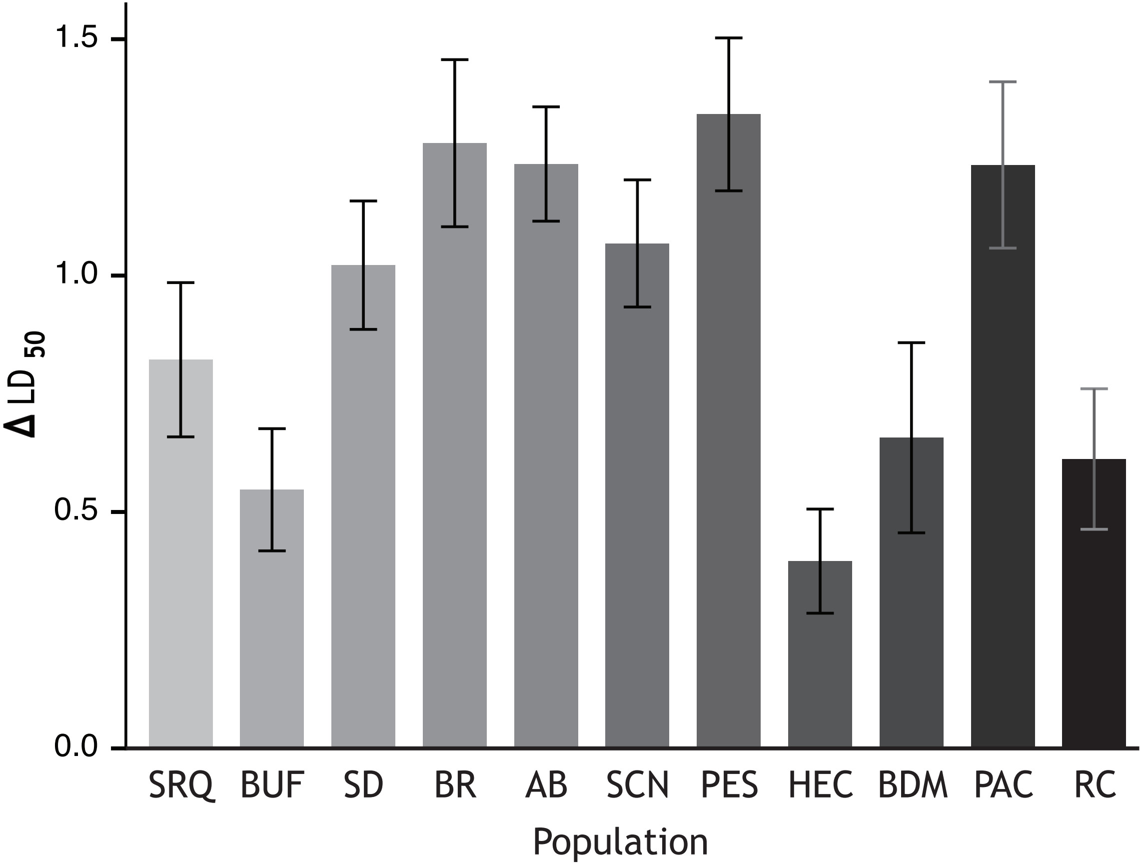


B. Thermal Limit by population

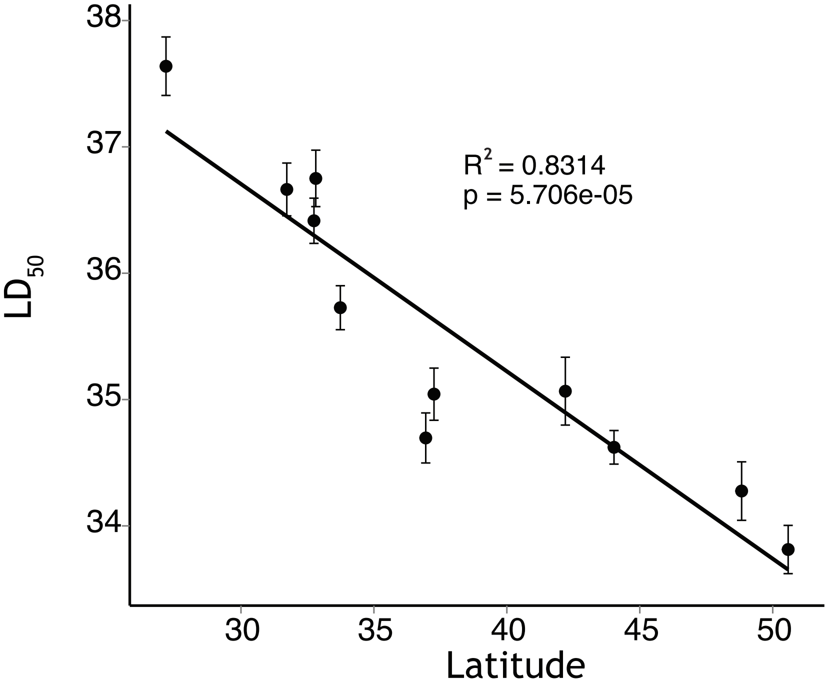
A. Change in LD50 vs. Change in LD10



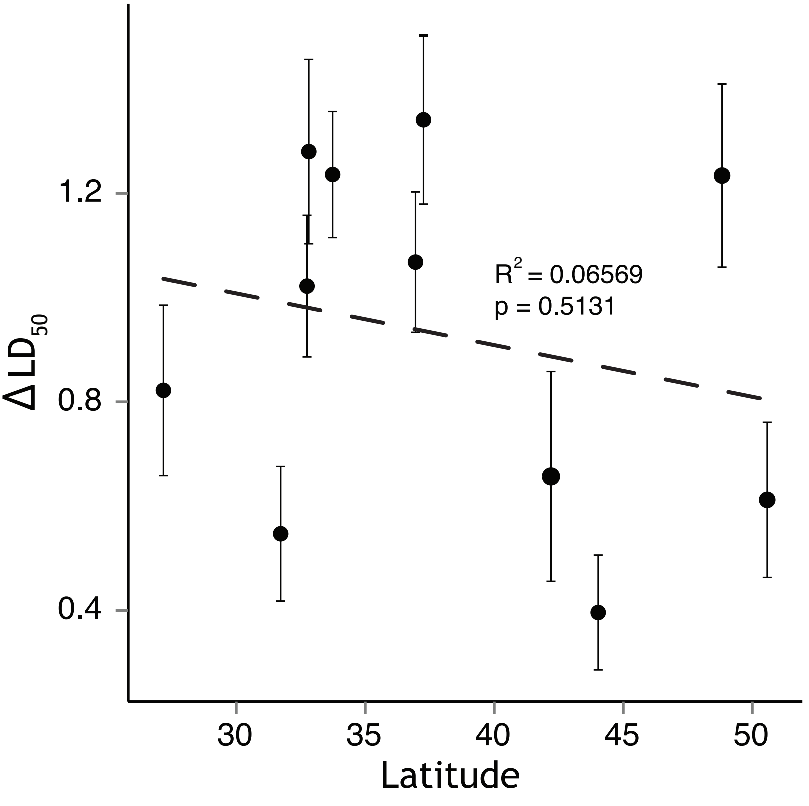
A. Change in Thermal Tolerance



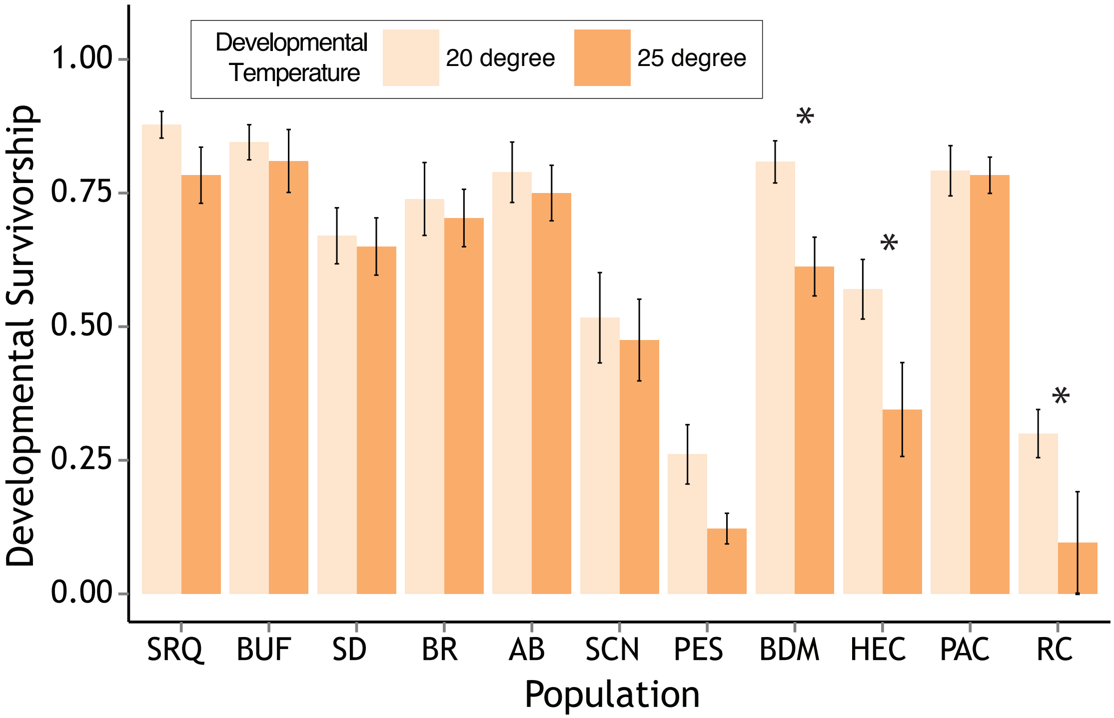
A. Genetic adaptation of thermal tolerance



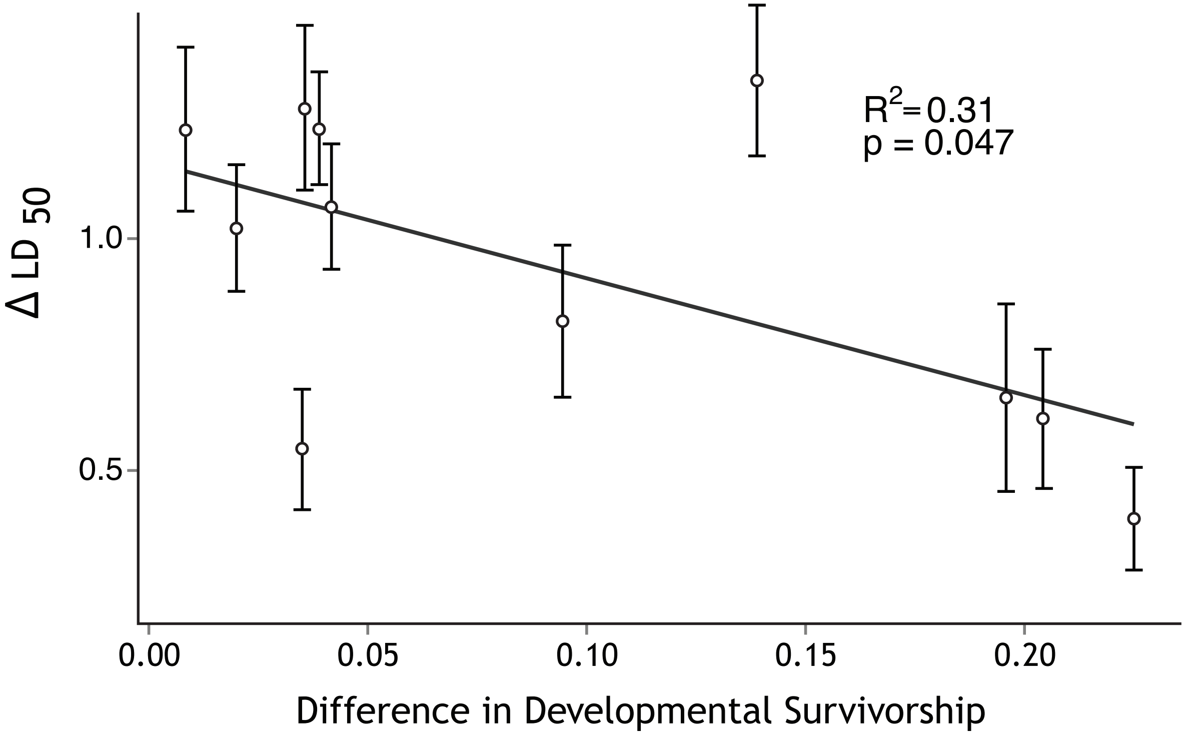
B. Adaptive phenotypic plasticity in thermal tolerance

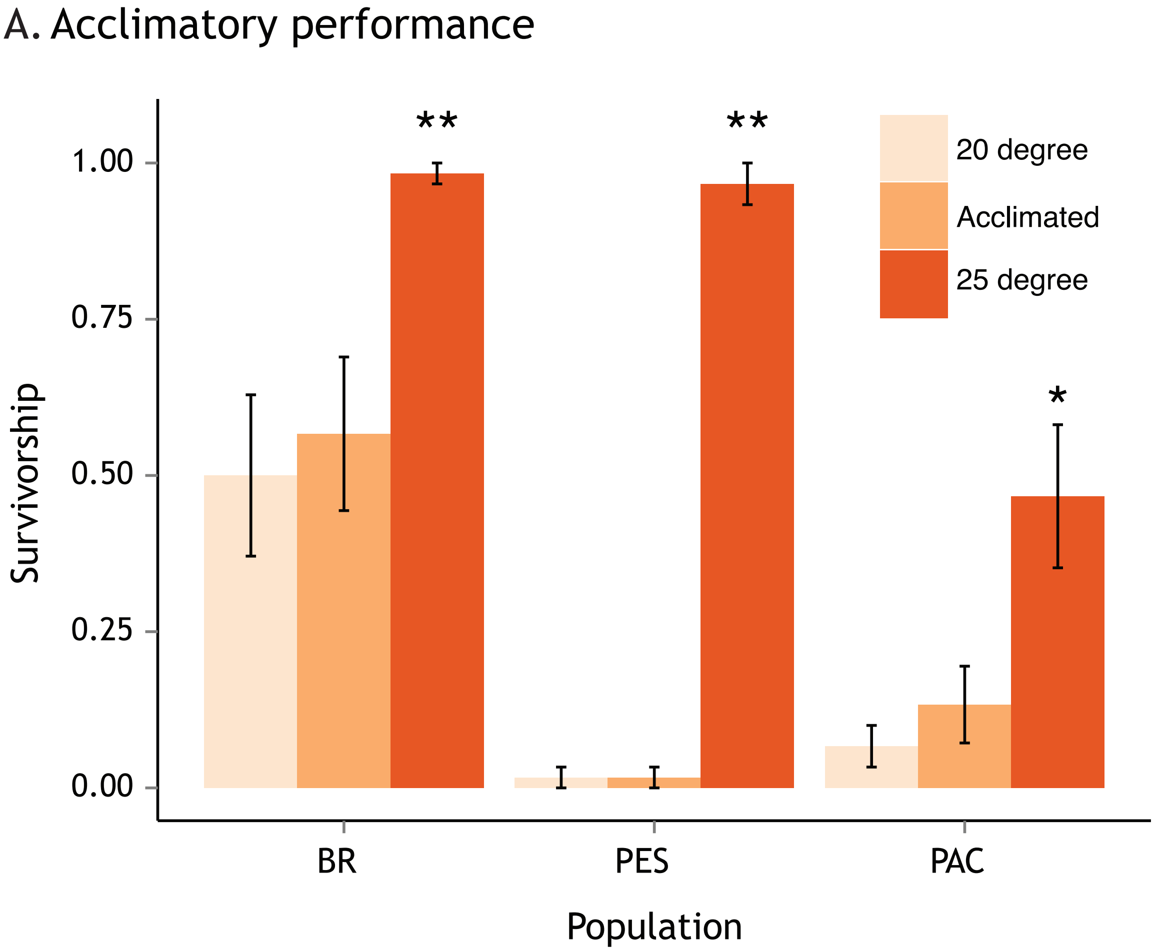


A. Developmental Survivorship by population

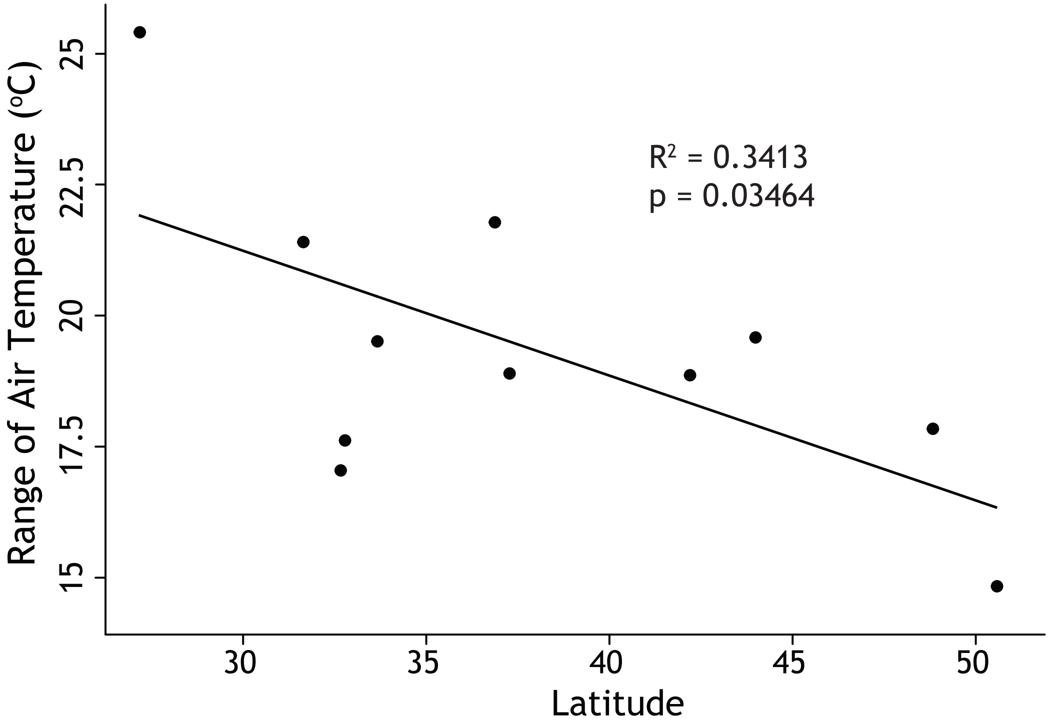


B. Developmental Survivorship and delta LD50




A. Acclimatory performance
Survivorship
1.00
0.75
0.50
0.25
0.00
**
**
*
20 degree
Acclimated
25 degree
BR
PES
PAC
Population

A. Annual temperature range vs. latitude



B. Annual mean temperature vs. latitude