Disparate patterns of thermal adaptation between life stages in temperate vs. tropical populations of *Drosophila melanogaster*

Brent L. Lockwood*, Tarun Gupta, and Rosemary Scavotto

Department of Biology, The University of Vermont, Burlington, VT 05405

*Corresponding author: Brent L. Lockwood
Email: Brent.Lockwood@uvm.edu

Abstract

Ectotherms may be threatened by increasing global temperatures, particularly if thermal adaptation is slower than climate change, as recent work has suggested. But estimates of evolutionary rates depend on the strength of natural selection, which may vary across life stages. Thus, depending on the life stage examined, comparisons of thermal tolerance among populations and species may yield inaccurate estimates of the rate at which thermal tolerance can evolve. Here we compared acute thermal tolerance in two life stages, adults and embryos, among populations of *Drosophila melanogaster* that span a broad range of thermal habitats across temperate sites in eastern North America and tropical sites around the globe. We report no variation in adult thermal tolerance among populations across this broad geographic range. In contrast, embryonic thermal tolerance was significantly higher in individuals from tropical sites compared to temperate sites. Embryonic thermal tolerance was similar among temperate populations, but among tropical populations embryonic thermal tolerance was positively correlated with maximum habitat temperature. We further report that embryos live closer to their upper thermal limits than adults—i.e., thermal safety margins are smaller for embryos than adults. F1 hybrid embryos from crosses between temperate and tropical populations had embryonic thermal tolerance that matched that of tropical embryos, suggesting phenotypic dominance of heat-tolerant alleles. Together our findings demonstrate that thermal tolerance readily evolves in the earliest and most thermally sensitive life stage and suggest that thermal selection may be more limited in the adult life stage. Disparate patterns of embryonic thermal tolerance between temperate and tropical populations may be the result of regional differences in seasonality.

Introduction

Extreme temperatures, which may be encountered at the edge of a species' geographic range (Hilbish et al., 2010) or episodically during the hottest or coldest days of the year (Hoffmann, 2010; Dowd et al., 2015; Buckley and Huey, 2016), can cause populations to experience mortality (Helmuth et al., 2002; Denny et al., 2006) and ultimately lead to thermal adaptation (Lenski and Bennett, 1993; Mongold et al., 1999; Hangartner and Hoffmann, 2015). However, recent work suggests that thermal adaptation of upper thermal limits may be evolutionarily constrained (Hoffmann et al., 2013; Schou et al., 2014; Hangartner and Hoffmann, 2015; Kristensen et al., 2015; van Heerwaarden et al., 2016), such that the evolution of increased heat tolerance may be a relatively slow process that takes many millions of years to achieve (Kellermann et al., 2012). If this is the case, global climate change, which has led to rapid increases in mean temperatures and the frequency of extreme thermal events (Katz and Brown, 1992; Meehl et al., 2000; Cai et al., 2014), may cause shifts in geographic distributions (Rank and Dahlhoff, 2002; Burrows et al., 2011; Thomas et al., 2012; Sunday et al., 2012) as populations may not be able to adapt fast enough to persist in hotter environments.

But evolutionary rates depend on several factors that may not be taken into account in estimates of thermal adaptive potential. In particular, adaptive rates depend on the strength of natural selection (Bennett et al., 1992; Rudolph et al., 2010), and studies that focus on thermal tolerance of mobile organisms or life stages may over estimate the degree to which these organisms encounter thermal selection in nature. In other words, thermal safety margins—i.e., the difference between upper thermal limits and maximum habitat temperature—may be larger than predicted because thermal environmental heterogeneity allows mobile organisms to avoid thermal extremes via behavioral thermoregulation (Dillon et al., 2009; Gunderson and Leal, 2012). To date, there have been relatively few studies that examine thermal adaptation in immobile organisms or life stages, particularly in the terrestrial realm (Angilletta et al., 2013; MacLean et al., 2016), and immobile organisms may represent ideal study systems to investigate the evolutionary potential of thermal tolerance. In support of this conjecture, broad scale patterns of thermal tolerance are more tightly correlated with habitat temperatures in marine systems than in terrestrial systems (Sunday et al., 2011), perhaps due to the more limited range of thermal microhabitats in the marine realm (Denny et al., 2011) that makes behavioral thermoregulation a less effective buffering mechanism.

Here we sought to compare adult and embryonic heat tolerance among populations of fruit flies, *Drosophila melanogaster*, from a broad range of thermal habitats across the world to ascertain the degree to which thermal selection has shaped the evolution of thermal tolerance across immobile vs. mobile life stages. Adult thermal tolerance has been extensively studied in natural populations of *D. melanogaster* —particularly in eastern Australia (Hoffmann et al., 2002; Sgro et al., 2010; Buckley and Huey, 2016)—but to a large extent, the thermal physiology of the early embryonic life stage of *D. melanogaster* has not been characterized in natural populations (Kristensen et al., 2015). Studies of lab-bred *D. melanogaster* have shown that early embryos (0 – 2 hours post-fertilization) are more thermally sensitive than later stages (Walter et

al., 1990), perhaps due to the reduced heat-shock response in early embryos (Graziosi et al., 1980; Welte et al., 1993). Thus, we compared heat tolerance of adults and early stage embryos to determine whether or not differences in thermal sensitivity, as well as mobility, lead to different patterns of thermal adaptation across life stages. The thermal environment of *D. melanogaster* can change rapidly (+18°C h^{-1}) and reach extreme values (> 40°C) (Feder et al., 1997). Therefore, we designed our thermal stress experiments to mimic sudden (acute) changes in temperature that are characteristic of the variable thermal environments that flies experience in nature (Terblanche et al., 2011). We report higher embryonic thermal tolerance in tropical (hotter) vs. temperate (cooler) populations but no difference in adult thermal tolerance, and thus we demonstrate that thermal selection likely varies across life stages. Moreover, our data suggest that there is significant adaptive potential for upper thermal limits in the earliest and most thermally sensitive life stage.

Results

Thermal tolerance and thermal safety margins across life stages

We found no difference in adult thermal tolerance among all sites (Figs. 1B and 1C), with an overall mean CT_{max} (± 95% C.I.) of 38.8 ± 0.45°C. Embryonic thermal tolerance (LT_{50}) did not differ among the three temperate sites but was significantly higher in tropical vs. temperate embryos (Figs. 1D and 1E). Overall, tropical embryos were more heat tolerant; the average LT_{50} was approximately 1°C higher in tropical embryos (35.8 ± 0.45°C) than in temperate embryos (34.88 ± 0.18°C). There was no significant relationship between adult CT_{max} and embryo LT_{50} (Fig. S1).

Thermal safety margins—i.e., the difference between thermal tolerance (CT_{max} or LT_{50}) and maximum habitat temperature (T_{max})—were consistently smaller for embryos than adults and smallest in the tropics. This pattern was consistent regardless of whether thermal safety margins were compared across latitude (Fig. 2A) or by region (temperate vs. tropical) (Fig. 2B).

Climate variability and thermal tolerance

While there is a consistent difference between our temperate and tropical sites in mean annual temperature, VT = 5.4°C, IN = 10.8°C, NC = 15.4°C, and all tropical = 26.1 ± 1.4°C (mean ± 95% C.I.), a principal components analysis of 19 Bioclim variables indicated that the greatest difference in climate among the collection locations was in temperature seasonality—defined as the yearly standard deviation in temperature. PC1 explained 95.6% of the variation in climate data among the collection sites, and this pattern largely characterized the differences in climate between temperate vs. tropical sites (Fig. S2). Temperature seasonality was the variable with the top component loading of PC1, explaining 98% of the variation in PC1. There was a significant negative relationship between PC1 and embryonic heat tolerance (Multiple least-squares linear regression with PC1 and PC2 as covariates, $F_{1,4} = 8.1474$, $P = 0.0462$), but this pattern was driven by the bimodal distribution of LT_{50}s between temperate vs. tropical sites and was not consistent with temperature seasonality within each geographic region (Fig. S3). PC2 explained

4% of the variation in climate data among collection sites, mostly characterizing the differences in climate among tropical sites (Fig. S2). Annual precipitation, precipitation of the wettest quarter, and precipitation of the wettest month were the top three component loadings of PC2, explaining 49%, 40%, and 8% of variation in PC2, respectively. But there was no significant relationship between PC2 and embryonic heat tolerance (Least-squares linear regression, $F_{1,4}$ = 0.367, P = 0.577).

Maximum temperature of the warmest month (i.e., maximum habitat temperature or T_{max}) spanned a range of 8.4°C among all sites, from 25.7°C in Vermont, USA (VT) to 34.1°C in Chiapas, MX (CH). While this variation in T_{max} explained only a small fraction of the variation in Bioclim variables among sites (0.0016% of the variation in PC1 and 0.00015% of the variation in PC2), previous studies have shown T_{max} to be correlated with adult heat tolerance (CT_{max}) among many species of *Drosophila* (Kellermann et al., 2012). In contrast to this previous work, our populations of *D. melanogaster* showed no significant relationship between adult heat tolerance and T_{max} (Fig. S4). However, the embryonic life stage exhibited a different pattern than the adults, and the relationship between embryonic heat tolerance and T_{max} was distinct between temperate and tropical regions (Fig. 3; ANCOVA, $F_{1,4}$ = 10.26, P = 0.0328). Among temperate populations there was a 6°C range in T_{max}, but this produced no correlated response in the thermal tolerance of embryos (Fig. 3). But among tropical populations, the approximate 4°C range in T_{max} corresponded to a positive relationship between embryonic thermal tolerance and T_{max} (Fig. 3).

Embryonic thermal tolerance in F1 progeny from Chiapas x Vermont

Offspring from reciprocal genetic crosses between the most heat tolerant tropical genotype (CH) and the least heat tolerant temperate genotype (VT-12) had thermal tolerances that closely resembled that of the heat tolerant CH genotype, regardless of the direction of the cross (Fig. 4). Embryonic LT_{50}s of F1 progeny of both crosses (CH♀ x VT♂ = 35.83°C and VT♀ x CH♂ = 35.80°C) were statistically indistinguishable from the LT_{50} of CH (36.24°C) but significantly higher than the LT_{50} of VT-12 (34.23°C; Fig. 4).

Discussion

Despite the potential for thermal adaptation across the broad range of thermal habitats represented in this study, our data suggest that thermal selection does not act equally across life stages in *D. melanogaster*. Rather, we provide evidence of adaptive variation in upper thermal limits in the thermally sensitive and immobile embryonic life stage but not in the more thermally tolerant and mobile adult stage. This is perhaps not surprising, given that lower thermal tolerance in early embryos translates into smaller thermal safety margins. Thus, we predict that embryos encounter lethal temperatures more frequently than adults, particularly because embryos lack the ability to behaviorally avoid thermally stressful conditions, and this likely drives divergence in embryonic thermal tolerance between temperate and tropical populations.

Recent estimates of divergence in adult thermal tolerance among populations of *D. melanogaster* have brought into question the degree of adaptive potential in upper thermal limits in this species, as comparisons of populations across latitude have yielded mixed results depending on assay methods (Sgro et al., 2010) and the laboratory in which thermal tolerance was measured (Hoffmann et al., 2002; Hoffmann, 2010; Buckley and Huey, 2016). Our estimates of *D. melanogaster* adult male CT_{max} are consistent with previous reports (Gilchrist et al., 1997; Chown et al., 2009; Kellermann et al., 2012), and while we report novel findings on the adaptation of embryonic thermal tolerance, our results are not unprecedented. Coyne et al. (Coyne et al., 1983) reported a similar discrepancy in thermal adaptation between mobile and immobile life stages among populations of *Drosophila pseudoobscura*—pupal thermal tolerance, but not adult thermal tolerance, was higher in populations from warmer locations. Thus, comparisons of adult (mobile stage) thermal tolerance among populations may not accurately predict the adaptive potential of upper thermal limits. Moreover, the interplay of population genetic factors in natural populations of *D. melanogaster* suggest that this species harbors a high level of genetic diversity (Karasov et al., 2010) and that natural selection has led to allelic divergence among populations across the genome (Fabian et al., 2012). In light of these trends in population genomics, and the adaptive variation in embryonic thermal tolerance presented in this study, it seems probable that there is significant natural variation of upper thermal limits in *D. melanogaster* but that this variation may only be revealed in the embryonic and other immobile life stages.

It is important to note that lab selection experiments in *D. melanogaster*, *Escherichia coli*, and marine copepods (*Tigriopus californicus*) that imposed strong selection on thermal tolerance reported significant potential for adaptation of upper thermal limits, but the response to selection eventually plateaued after many generations, presumably when standing genetic diversity had been exhausted (Huey et al., 1991; Gilchrist et al., 1997; Gilchrist and Huey, 1999; Rudolph et al., 2010; Kelly et al., 2012; Hangartner and Hoffmann, 2015). Thus, while there may likely be potential for adaptation of upper thermal limits, responses to thermal selection may not occur indefinitely in the absence of new mutational variation.

This study characterizes thermal tolerance among populations that span a large portion of the *D. melanogaster* biogeographic range in the northern hemisphere, and while there is clear evidence of adaptation of embryonic thermal tolerance between temperate and tropical regions, the patterns of thermal adaptation are not consistent within each region. Tropical embryos sampled from locations with higher T_{max} showed higher thermal tolerances, yet temperate populations did not follow this trend. Why were there no observed differences in embryonic thermal tolerance among temperate populations when temperate sites spanned a broader range of thermal habitats than tropical populations? It is possible that gene flow between Vermont, Indiana, and North Carolina overwhelms local adaptation, but recent studies show evidence of adaptive divergence among *D. melanogaster* populations in eastern North America (Fabian et al., 2012; Bergland et al., 2016; Machado et al., 2016). Therefore, a more likely explanation is that seasonal fluctuations in the activity of temperate populations (Cogni et al., 2014; Bergland et al.,

2014), may limit the frequency at which temperate embryos encounter thermal selection. In addition, spatial and temporal microclimatic variability in temperate sites may provide more choices for females to lay their eggs at permissive temperatures (Allemand and David, 1976; Dahlgaard et al., 2001; Huey and Pascual, 2009; Dillon et al., 2009). We also note that our data constitute thermal tolerances of multiple isofemale lines from each of the three temperate sites but just one isofemale line from each of the five tropical sites. Thus, we have not captured the full range of genetic variation at each tropical site. The fact that the tropical flies originated from geographically distant locations suggests that our observed differences in embryonic thermal tolerance between temperate and tropical populations represent consistent patterns of thermal adaptation. However, our observation that embryonic thermal tolerance positively correlates with maximum temperature at tropical sites is a result that warrants further investigation. It remains to be determined whether or not this pattern will hold when a greater sample of genetic diversity is surveyed at these topical locations.

Investigations into the genetic basis of variation in thermal tolerance in natural populations have yielded somewhat conflicting results, depending on the species (Pereira et al., 2014; Sato et al., 2015) and the way thermal tolerance was measured (Gilchrist and Huey, 1999; Mitchell and Hoffmann, 2010; Hangartner and Hoffmann, 2015). In this study, embryonic LT_{50} spanned a range of approximately 2°C among all genotypes, and crosses between genotypes at the high and low ends of this range produced F1 progeny that resembled the phenotype of the heat-tolerant parent. This result indicates phenotypic dominance of heat-tolerant alleles, at least for Chiapas vs. Vermont-12 embryos. This is a surprising result for two reasons. First, this inheritance pattern is not expected because thermal physiological traits are complex and depend on multiple interacting factors, such as lipid membrane composition (Dahlhoff and Somero, 1993; Cooper et al., 2012; Cooper et al., 2014), protein stability (Lockwood and Somero, 2012; Fields et al., 2015; Leuenberger et al., 2017), and transcriptional and translational responses (Leemans et al., 2000; Lockwood et al., 2010; Tomanek and Zuzow, 2010; Chen et al., 2015; Kristensen et al., 2016). Accordingly, previous work has shown that *D. melanogaster* adult and larval thermal tolerance are quantitative traits influenced by at least 7 gene loci (Morgan and Mackay, 2006; Sambucetti et al., 2013). Similarly in species of marine invertebrates, thermal tolerance appears to have a polygenic basis, as hybrids often exhibit thermal tolerances that are intermediate between the parents (Braby and Somero, 2006). But this is not the case for *D. melanogaster* embryonic thermal tolerance as we have characterized it here. Second, because we examined early *D. melanogaster* embryos at 0 – 1 h post-fertilization (hpf), and zygotic genome activation has been reported to not begin until approximately 2 hpf in this species (Tadros and Lipshitz, 2009; Blythe and Wieschaus, 2015), we expected thermal tolerance to be determined by maternal effects. Instead, we found embryonic thermal tolerance to match that of the Chiapas strain regardless of maternal genotype. This suggests that either (1) the zygotic genome is being activated earlier than expected in response to heat shock (Graziosi et al., 1980), which would reveal adaptive variation in zygotic gene expression, or (2) that the effect is mediated at the level of the chromosomes, perhaps due to thermally-induced DNA damage (Yao and Somero, 2012)

that differentially affects different genotypes (Svetec et al., 2016). Either way, the unknown genetic basis of embryonic thermal tolerance warrants future study.

Materials and methods

Fly strains

We obtained 19 isofemale genetic lines that were collected from temperate locations in the USA as a generous gift from B.S. Cooper and K.L. Montooth: 6 lines from Raleigh, NC (NC); 5 lines from Beasley Orchard, IN (IN); and 8 lines from East Calais, VT (VT). The creation of these lines has been previously described (Cooper et al., 2014). We obtained 5 isofemale lines from the Drosophila Species Stock Center at the University of California, San Diego that were collected from tropical locations around the world: 1 line each from Accra, Ghana (GH); Mumbai, India (MU); Guam, USA (GU); Chiapas, Mexico (CH); and Monkey Hill, St. Kitts (SK). Geographic coordinates of collection locations and stock numbers of isofemale lines are provided in Supplementary Table S1. We maintained flies under common-garden conditions on cornmeal-yeast-molasses medium at 25°C on a 12:12 light cycle for at least two generations prior to measuring thermal tolerance.

Adult critical thermal maximum (CT_{max})

We assayed thermal tolerance of adult male flies by measuring the temperature at which flies incurred a loss of motor response along a heat ramp—i.e., the critical thermal maximum (CT_{max}). 3 to 5 day-old adult male flies were individually placed into glass vials with rubber stoppers and submerged in a water bath that was programmed to increase 0.1°C min^{-1} upwards from 25°C. We chose this rate of temperature increase based on previously published studies (Chown et al., 2009; Sgro et al., 2010; Kellermann et al., 2012) and to mimic the variable thermal environments that flies encounter in nature (Terblanche et al., 2011). Flies were regularly checked for responsiveness along the heat ramp by gently tapping the vial, and the temperature at which a fly lost the ability to move was recorded. We scored CT_{max} for each genotype via a least-squares regression model of the logistic equation among 10 flies per genotype and extrapolated CT_{max} from the inflection points of the logistic curves. We conducted these curve fitting analyses in GraphPad Prism 7 for Mac OS X (GraphPad Software, La Jolla, CA).

Embryonic thermal tolerance (LT_{50})

We assayed embryonic thermal tolerance by measuring survival (hatching success) of early stage embryos, 0 to 1 h post-fertilization, exposed to a 45-minute heat treatment across a range of temperatures, from 25°C to 42°C. We chose this assay method because embryos do not possess behavioral characteristics that would permit the assessment of thermal tolerance via CT_{max}, and measuring hatching success allowed us to overcome this obstacle. Previous studies conducted in a wide range of species have shown that temperature adaptive variation in survival following heat stress follows the same trends as CT_{max} (Huey et al., 1991; Gilchrist et al., 1997; Stillman and Somero, 2000; Somero, 2002; Stenseng et al., 2005; Braby and Somero, 2006; Miller and

Stillman, 2012; Dowd and Somero, 2013). We designed our heat treatments to mimic sudden increases in temperature that frequently occur in nature where the temperature of necrotic fruit can increase rapidly on hot days (Feder et al., 1997; Terblanche et al., 2011). 3 to 5 day-old adult flies were allowed to mate and lay eggs on grape juice agar plates for 1 h at 25°C. Egg plates were then wrapped in Parafilm, submerged in a water bath, and heat shocked for 45 minutes. The rate of temperature change averaged +0.4°C min^{-1}. This rate of increase is within the range of measured rates of change in nature (Feder et al., 1997). Following heat shock, 20 eggs were transferred on a piece of grape juice agar to fresh food vials and placed at 25°C. Hatching success was scored as the proportion of larvae that successfully hatched by 48 h. We conducted 4 to 6 replicate treatments at each of 9 temperatures (25°C, 28°C, 30°C, 32°C, 34°C, 36°C, 38°C, 40°C, and 42°C) for each genotype. We used these data to calculate the lethal temperature at which 50% of the embryos failed to hatch (LT_{50}) via a least-squares regression model of the logistic equation. In our logistic model, we allowed the y-intercept to vary between 0 and 1 and extrapolated the LT_{50} from the inflection point of the logistic curve fit. This approach allowed us to infer thermal tolerance independently from other confounding factors that may influence the measurement of hatching success, such as the presence of unfertilized eggs. We conducted these curve fitting analyses in GraphPad Prism 7.

PCA of bioclimatic variables

We examined the correspondence of adult and embryonic thermal tolerance to local climate conditions by examining the variation among our collection sites in 19 bioclimatic (Bioclim) variables. Bioclim variables represent biologically meaningful climate predictors that are derived from monthly temperature and rainfall data from the years 1960 to 1990 (Busby, 1991). We downloaded Bioclim variables from the WorldClim database (Hijmans et al., 2005) (www.worldclim.org) that corresponded to the GPS coordinates of the collection sites of each population (see Table S1). Because Bioclim variables tend to covary, we used a principal components analysis (PCA) to characterize the major axes of variation in climate among our sites. We then conducted a multiple least-squares regression analysis to assess the degree to which variation in thermal tolerance could be explained by the primary principal components of these climate data. We conducted these analyses in R version 3.3.2 (Team, 2016).

Statistical comparisons of thermal tolerance and thermal safety margins

We compared adult (CT_{max}) and embryonic (LT_{50}) thermal tolerances among temperate sites (VT, IN, and NC) and all tropical sites pooled together (CH, SK, GH, MU, and GU) with ANOVA. Pairwise differences were assessed with Tukey's multiple comparison post-hoc test. We report our data as mean values with 95% confidence intervals. We calculated thermal safety margins as the difference between thermal tolerance (adult CT_{max} or embryo LT_{50}) and maximum temperature of the warmest month (T_{max}) at each site (see above for description of Bioclim data). Fine-scale spatial temperature data are not available for these collection sites, but while T_{max} may not perfectly match the thermal environment experienced by flies, variation in T_{max} should reflect

relative differences in the thermal environments among locations. We compared thermal safety margins across latitude, as in Kellerman et al. (Kellermann et al., 2012), between adults and embryos using non-linear least-squares regression followed by the extra sum-of-squares F-test to compare model fits. In a separate analysis, we assessed the main effects of region (temperate vs. tropical), life stage (adult vs. embryo), and their interaction on thermal safety margins via a 2-way ANOVA. Least-squares linear regression was used to assess the relationship between embryonic thermal tolerance and significant climate variables. ANCOVA was used to assess the difference in slopes of regression lines fit to data from temperate vs. tropical sites. We assessed the genetic basis of differences in embryonic thermal tolerance by conducting reciprocal crosses between the two parental strains that had the highest and lowest LT_{50}, Chiapas, MX (CH) and Vermont, USA strain #12 (VT-12), respectively, and measured thermal tolerance of F1 progeny. We used logistic models to fit the hatching success data, as described above, and compared LT_{50}s of the parental strains and their F1 progeny by an extra sum-of-squares F-test of the extrapolated LT_{50}s. We conducted these analyses in GraphPad Prism 7.

Acknowledgements

We thank Kristi Montooth and Cole Julick for generously providing fly stocks. We also thank Andrew Nguyen for R code that was helpful in generating the map shown in Figure 1. This work was funded by the University of Vermont.

References

Allemand, R. and David, J. R. (1976). The circadian rhythm of oviposition in *Drosophila melanogaster*: a genetic latitudinal cline in wild populations. *Experientia* **32**, 1403–1404.

Angilletta, M. J., Zelic, M. H., Adrian, G. J., Hurliman, a. M. and Smith, C. D. (2013). Heat tolerance during embryonic development has not diverged among populations of a widespread species (*Sceloporus undulatus*). *Conserv. Physiol.* **1**, cot018-cot018.

Bennett, A. F., Lenski, R. E. and Mittler, J. E. (1992). Evolutionary Adaptation to Temperature . I . Fitness Responses of *Escherichia coli* to Changes in its Thermal Environment. *Evolution (N. Y).* **46**, 16–30.

Bergland, A. O., Behrman, E. L., O'Brien, K. R., Schmidt, P. S. and Petrov, D. a. (2014). Genomic Evidence of Rapid and Stable Adaptive Oscillations over Seasonal Time Scales in *Drosophila*. *PLoS Genet.* **10**, e1004775.

Bergland, A. O., Tobler, R., Gonzalez, J., Schmidt, P. and Petrov, D. (2016). Secondary contact and local adaptation contribute to genome-wide patterns of clinal variation in *Drosophila melanogaster*. *Mol. Ecol.* **25**, 1157–1174.

Blythe, S. A. and Wieschaus, E. F. (2015). Coordinating Cell Cycle Remodeling with Transcriptional Activation at the *Drosophila* MBT. *Curr. Top. Dev. Biol.* **113**, 113–148.

Braby, C. E. and Somero, G. N. (2006). Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *J. Exp. Biol.* **209**, 2554–2566.

Buckley, L. B. and Huey, R. B. (2016). How Extreme Temperatures Impact Organisms and the Evolution of their Thermal Tolerance. *Integr. Comp. Biol.* 1–12.

Burrows, M. T., Schoeman, D. S., Buckley, L. B., Moore, P., Poloczanska, E. S., Brander, K. M., Brown, C., Bruno, J. F., Duarte, C. M., Halpern, B. S., et al. (2011). The pace of shifting climate in marine and terrestrial ecosystems. *Science (80-.).* **334**, 652–655.

Busby, J. R. (1991). BIOCLIM-a bioclimate analysis and prediction system. *Plant Prot. Q.*

Cai, W., Borlace, S., Lengaigne, M., van Rensch, P., Collins, M., Vecchi, G., Timmermann, A., Santoso, A., McPhaden, M. J., Wu, L., et al. (2014). Increasing frequency of extreme El Niño events due to greenhouse warming. *Nat. Clim. Chang.* **5**, 1–6.

Chen, J., Nolte, V. and Schlotterer, C. (2015). Temperature-Related Reaction Norms of Gene Expression: Regulatory Architecture and Functional Implications. *Mol. Biol. Evol.*

Chown, S. L., Jumbam, K. R., Sørensen, J. G. and Terblanche, J. S. (2009). Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. *Funct. Ecol.* **23**, 133–140.

Cogni, R., Kuczynski, C., Koury, S., Lavington, E., Behrman, E. L., O'Brien, K. R., Schmidt, P. S. and Eanes, W. F. (2014). The Intensity of Selection Acting on the Couch Potato Gene - Spatial-Temporal Variation in a Diapause Cline. *Evolution (N. Y).* **68**, 538–548.

Cooper, B. S., Hammad, L. a., Fisher, N. P., Karty, J. a. and Montooth, K. L. (2012). In a variable thermal environment selection favors greater plasticity of cell membranes in drosophila melanogaster. *Evolution (N. Y).* **66**, 1976–1984.

Cooper, B. S., Hammad, L. a and Montooth, K. L. (2014). Thermal adaptation of cellular membranes in natural populations of *Drosophila melanogaster*. *Funct. Ecol.* **28**, 886–894.

Coyne, J. a., Bundgaard, J. and Prout, T. (1983). Geographic Variation of Tolerance to Environmental Stress in *Drosophila pseudoobscura*. *Am. Nat.* **122**, 474.

Dahlgaard, J., Hasson, E. and Loeschcke, V. (2001). Behavioral differentiation in oviposition

activity in *Drosophila buzzatii* from highland and lowland populations in Argentina: plasticity or thermal adaptation? *Evolution (N. Y).* **55**, 738–47.

Dahlhoff, E. and Somero, G. N. (1993). Effects of temperature on mitochondria from Abalone (genus *Haliotis*) - adaptive plasticity and its limits. *J. Exp. Biol.* **185**, 151–168.

Denny, M. W., Miller, L. P. and Harley, C. D. G. (2006). Thermal stress on intertidal limpets: long-term hindcasts and lethal limits. *J. Exp. Biol.* **209**, 2420–2431.

Denny, M. W., Dowd, W. W., Bilir, L. and Mach, K. J. (2011). Spreading the risk: Small-scale body temperature variation among intertidal organisms and its implications for species persistence. *J. Exp. Mar. Bio. Ecol.* **400**, 175–190.

Dillon, M. E., Wang, G., Garrity, P. a and Huey, R. B. (2009). Review: Thermal preference in *Drosophila*. *J. Therm. Biol.* **34**, 109–119.

Dowd, W. W. and Somero, G. N. (2013). Behavior and survival of *Mytilus* congeners following episodes of elevated body temperature in air and seawater. *J. Exp. Biol.* **216**, 502–14.

Dowd, W. W., King, F. A. and Denny, M. W. (2015). Thermal variation, thermal extremes and the physiological performance of individuals. *J. Exp. Biol.* **218**, 1956–1967.

Fabian, D. K., Kapun, M., Nolte, V., Kofler, R., Schmidt, P. S., Schlötterer, C. and Flatt, T. (2012). Genome-wide patterns of latitudinal differentiation among populations of *Drosophila melanogaster* from North America. *Mol. Ecol.* **21**, 4748–69.

Feder, M. E., Blair, N. and Figueras, H. (1997). Natural thermal stress and heat shock protein expression in *Drosophila* larvae and pupae. *Funct. Ecol.* **11**, 90–100.

Fields, P. A., Dong, Y., Meng, X. and Somero, G. N. (2015). Adaptations of protein structure and function to temperature: there is more than one way to "skin a cat." *J. Exp. Biol.* **218**, 1801–1811.

Gilchrist, G. W. and Huey, R. B. (1999). The direct response of *Drosophila melanogaster* to selection on knockdown temperature. *Heredity.* **83 (Pt 1)**, 15–29.

Gilchrist, G. W., Huey, R. and Partridge, L. (1997). Thermal Sensitivity of *Drosophila melanogaster*: Evolutionary Responses of Adults and Eggs to Laboratory Natural Selection at Different Temperatures. *Physiol. Zool.* **70**, 403–414.

Graziosi, G., Micali, F., Marzari, R., de Cristini, F. and Savoini, A. (1980). Variability of response of early *Drosophila* embryos to heat shock. *J. Exp. Zool.* **214**, 141–145.

Gunderson, A. R. and Leal, M. (2012). Geographic variation in vulnerability to climate warming in a tropical Caribbean lizard. *Funct. Ecol.* **26**, 783–793.

Hangartner, S. and Hoffmann, A. a. (2015). Evolutionary potential of multiple measures of upper thermal tolerance in *Drosophila melanogaster*. *Funct. Ecol.* **30**, n/a-n/a.

Helmuth, B., Harley, C. D. G., Halpin, P. M., O'Donnell, M., Hofmann, G. E. and Blanchette, C. A. (2002). Climate change and latitudinal patterns of intertidal thermal stress. *Science (80-.).* **298**, 1015–1017.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G. and Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**, 1965–1978.

Hilbish, T. J., Brannock, P. M., Jones, K. R., Smith, A. B., Bullock, B. N., Wethey, D. S. and Smith, AB (2010). Historical changes in the distributions of invasive and endemic marine invertebrates are contrary to global warming predictions: the effects of decadal climate oscillations. *J. Biogeogr.* **37**, 423–431.

Hoffmann, A. A. (2010). Physiological climatic limits in *Drosophila*: patterns and implications. *J. Exp. Biol.* **213**, 870–880.

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

Hoffmann, A. A., Chown, S. L. and Clusella-trullas, S. (2013). Upper thermal limits in terrestrial ectotherms: how constrained are they? *Funct. Ecol.* **27**, 934–949.

Huey, R. B. and Pascual, M. (2009). Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline. *Ecology* **90**, 1715–1720.

Huey, R. B., Patridge, L. and Fowler, K. (1991). Thermal sensitivity of *Drosophila melanogaster* responds rapidly to laboratory natural selection. *Evolution (N. Y).* **45**, 751–756.

Karasov, T., Messer, P. W. and Petrov, D. a (2010). Evidence that adaptation in *Drosophila* is not limited by mutation at single sites. *PLoS Genet.* **6**, e1000924.

Katz, R. W. and Brown, B. G. (1992). Extreme events in a changing climate: Variability is more important than averages. *Clim. Change* **21**, 289–302.

Kellermann, V., Overgaard, J., Hoffmann, A. a, Fløjgaard, C., Svenning, J.-C. and Loeschcke, V. (2012). Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 16228–33.

Kelly, M. W., Sanford, E. and Grosberg, R. K. (2012). Limited potential for adaptation to climate change in a broadly distributed marine crustacean. *Proc. R. Soc. B-Biological Sci.* **279**, 349–356.

Kristensen, T. N., Overgaard, J., Lassen, J., Hoffmann, A. a. and Sgrò, C. (2015). Low evolutionary potential for egg-to-adult viability in *Drosophila melanogaster* at high temperatures. *Evolution (N. Y).* **69**, 803–814.

Kristensen, T. N., Kjeldal, H., Schou, M. F. and Nielsen, J. L. (2016). Proteomic data reveals a physiological basis for costs and benefits associated with thermal acclimation. *J. Exp. Biol.* in press.

Leemans, R., Egger, B., Loop, T., Kammermeier, L., He, H., Hartmann, B., Certa, U., Hirth, F. and Reichert, H. (2000). Quantitative transcript imaging in normal and heat-shocked *Drosophila* embryos by using high-density oligonucleotide arrays. *Proc Natl Acad Sci U S A* **97**, 12138–12143.

Lenski, R. E. and Bennett, A. F. (1993). Evolutionary Response of *Escherichia coli* to Thermal Stress. *Am. Nat.* **142**, S47–S64.

Leuenberger, P., Ganscha, S., Kahraman, A., Cappelletti, V., Boersema, P. J., von Mering, C., Claassen, M. and Picotti, P. (2017). Cell-wide analysis of protein thermal unfolding reveals determinants of thermostability. *Science (80-.).* **355**, eaai7825.

Lockwood, B. L. and Somero, G. N. (2012). Functional determinants of temperature adaptation in enzymes of cold- versus warm-adapted mussels (Genus *Mytilus*). *Mol. Biol. Evol.* **29**, 3061–70.

Lockwood, B. L., Sanders, J. G. and Somero, G. N. (2010). Transcriptomic responses to heat-stress in invasive and native blue mussels (genus *Mytilus*): molecular correlates of invasive success. *J. Exp. Biol.* **213**, 3548–3558.

Machado, H. E., Bergland, A. O., O'Brien, K. R., Behrman, E. L., Schmidt, P. S. and Petrov, D. A. (2016). Comparative population genomics of latitudinal variation in *D. simulans* and *D. melanogaster*. *Mol. Ecol.*

MacLean, H. J., Higgins, J. K., Buckley, L. B. and Kingsolver, J. G. (2016). Geographic divergence in upper thermal limits across insect life stages: does behavior matter?

Oecologia.

Meehl, G. a., Zwiers, F., Evans, J., Knutson, T., Mearns, L. and Whetton, P. (2000). Trends in extreme weather and climate events: Issues related to modeling extremes in projections of future climate change. *Bull. Am. Meteorol. Soc.* **81**, 427–436.

Miller, N. a. and Stillman, J. H. (2012). Neural Thermal Performance in Porcelain Crabs, Genus *Petrolisthes*. *Physiol. Biochem. Zool.* **85**, 29–39.

Mitchell, K. a. and Hoffmann, A. a. (2010). Thermal ramping rate influences evolutionary potential and species differences for upper thermal limits in *Drosophila*. *Funct. Ecol.* **24**, 694–700.

Mongold, J. A., Bennett, A. F. and Lenski, R. E. (1999). Evolutionary adaptation to temperature. VII. Extension of the upper thermal limit of *Escherichia coli*. *Evolution.* **53**, 386–394.

Morgan, T. and Mackay, T. (2006). Quantitative trait loci for thermotolerance phenotypes in *Drosophila melanogaster*. *Heredity.* **96**, 232–242.

Pereira, R. J., Barreto, F. S. and Burton, R. S. (2014). Ecological novelty by hybridization: Experimental evidence for increased thermal tolerance by transgressive segregation in tigriopus californicus. *Evolution.* **68**, 204–215.

Rank, N. E. and Dahlhoff, E. P. (2002). Allele frequency shifts in response to climate change and physiological consequences of allozyme variation in a montane insect. *Evolution.* **56**, 2278–2289.

Rudolph, B., Gebendorfer, K. M., Buchner, J. and Winter, J. (2010). Evolution of *Escherichia coli* for Growth at High Temperatures. *J. Biol. Chem.* **285**, 19029–19034.

Sambucetti, P., Scannapieco, a C., Loeschcke, V. and Norry, F. M. (2013). Heat-stress survival in the pre-adult stage of the life cycle in an intercontinental set of recombinant inbred lines of *Drosophila melanogaster*. *J. Exp. Biol.* **216**, 2953–9.

Sato, A., Kawashima, T., Fujie, M., Hughes, S., Satoh, N. and Shimeld, S. M. (2015). Molecular basis of canalization in an ascidian species complex adapted to different thermal conditions. *Sci. Rep.* **5**, 1–9.

Schou, M. F., Kristensen, T. N., Kellermann, V., Schl??tterer, C. and Loeschcke, V. (2014). A Drosophila laboratory evolution experiment points to low evolutionary potential under increased temperatures likely to be experienced in the future. *J. Evol. Biol.* **27**,.

Sgro, C. M., Overgaard, J., Kristensen, T. N., Mitchell, K. A., Cockerell, F. E. and 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.

Somero, G. N. (2002). Thermal Physiology and Vertical Zonation of Intertidal Animals: Optima, Limits, and Costs of Living. *Integr. Comp. Biol.* **42**, 780–789.

Stenseng, E., Braby, C. E., Somero, G. N. and Anonymous (2005). Evolutionary and acclimation-induced variation in the thermal limits of heart function in congeneric marine snails (Genus *Tegula*): Implications for vertical zonation. *Biol. Bull. (Woods Hole)* **208**, 138–144.

Stillman, J. H. and 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.

Sunday, J. M., Bates, A. E. and Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proc. Biol. Sci.* **278**, 1823–30.

526 **Sunday, J. M., Bates, A. E. and Dulvy, N. K.** (2012). Thermal tolerance and the global
527 redistribution of animals. *Nat. Clim. Chang.* **2**, 686–690.
528 **Svetec, N., Cridland, J. M., Zhao, L. and Begun, D. J.** (2016). The Adaptive Significance of
529 Natural Genetic Variation in the DNA Damage Response of *Drosophila melanogaster*.
530 *PLOS Genet.* **12**, e1005869.
531 **Tadros, W. and Lipshitz, H. D.** (2009). The maternal-to-zygotic transition: a play in two acts.
532 *Development* **136**, 3033–42.
533 **Team, R. C.** (2016). *R: A language and environment for statistical computing*. Vienna, Austria:
534 R Foundation for Statistical Computing.
535 **Terblanche, J. S., Hoffmann, A. A., Mitchell, K. A., Rako, L., le Roux, P. C. and Chown, S.**
536 **L.** (2011). Ecologically relevant measures of tolerance to potentially lethal temperatures. *J.*
537 *Exp. Biol.* **214**, 3713–3725.
538 **Thomas, M. K., Kremer, C. T., Klausmeier, C. a and Litchman, E.** (2012). A global pattern
539 of thermal adaptation in marine phytoplankton. *Science (80-.).* **338**, 1085–1088.
540 **Tomanek, L. and Zuzow, M. J.** (2010). The proteomic response of the mussel congeners
541 *Mytilus galloprovincialis* and *M. trossulus* to acute heat stress: implications for thermal
542 tolerance limits and metabolic costs of thermal stress. *J. Exp. Biol.* **213**, 3559–3574.
543 **van Heerwaarden, B., Kellermann, V. and Sgrò, C. M.** (2016). Limited scope for plasticity to
544 increase upper thermal limits. *Funct. Ecol.*
545 **Walter, M. F., Biessmann, H. and Petersen, N. S.** (1990). Heat shock causes the collapse of
546 the intermediate filament cytoskeleton in *Drosophila* embryos. *Dev. Genet.* **11**, 270–279.
547 **Welte, M. A., Tetrault, J. M., Dellavalle, R. I. P. and Lindquist, S. L.** (1993). A new method
548 for manipulating transgenes: engineering heat tolerance in a complex, multicellular
549 organism. *Curr. Biol.* **3**, 842–853.
550 **Yao, C.-L. and Somero, G. N.** (2012). The impact of acute temperature stress on hemocytes of
551 invasive and native mussels (*Mytilus galloprovincialis* and *Mytilus californianus*): DNA
552 damage, membrane integrity, apoptosis and signaling pathways. *J. Exp. Biol.* **215**, 4267–77.
553
554
555

Figures and Tables

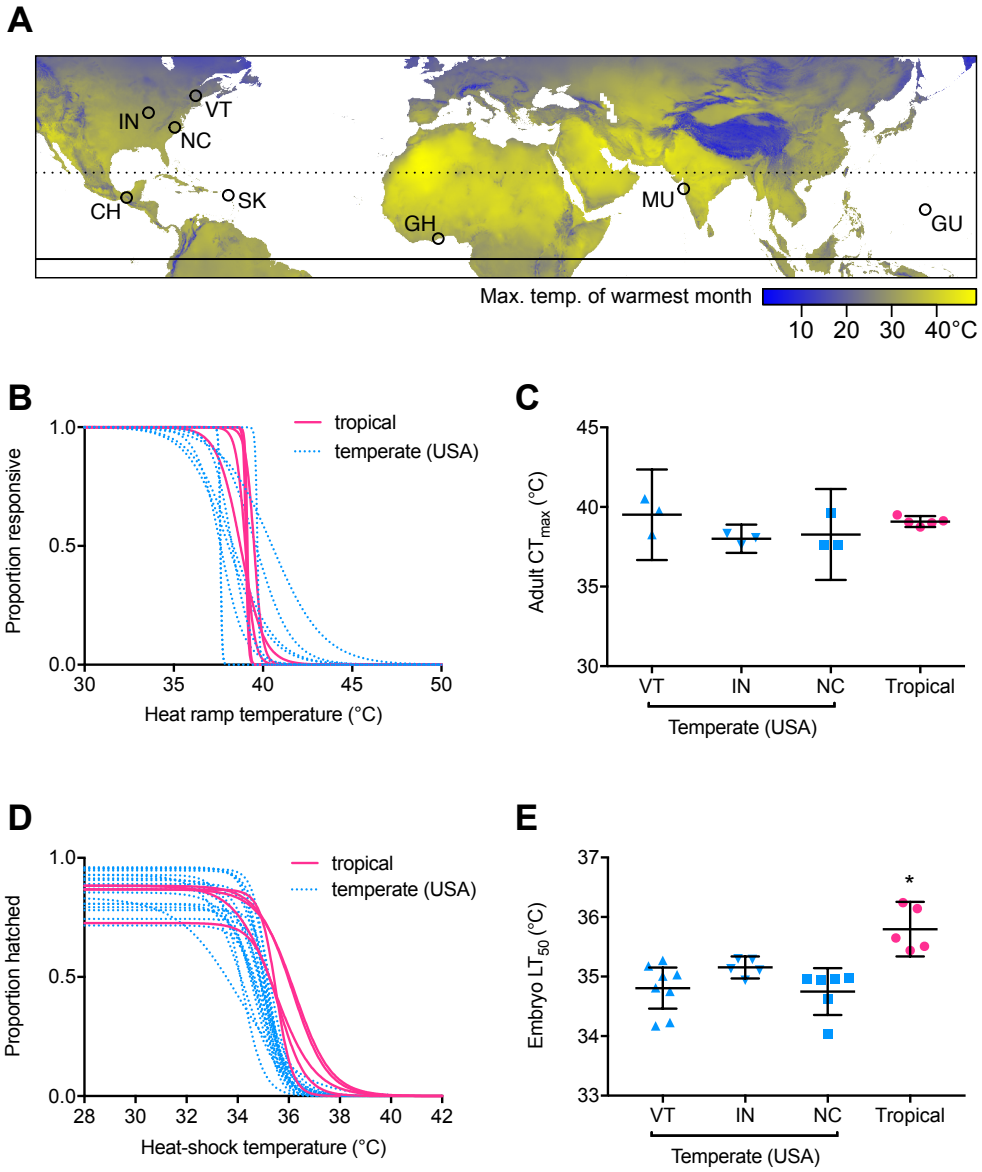


Figure 1.

Flies from different thermal environments around the world exhibited differences in embryonic thermal tolerance but not adult thermal tolerance.

(**A**) Sampling locations of isofemale lines. Color indicates maximum temperature of warmest month (T_{max}), extrapolated from the WoldClim database. Tropical populations lie between the Equator (solid horizontal line) and the Tropic of Cancer (dotted horizontal line) and were sampled in Chiapas, Mexico (CH), Saint Kitts (SK), Accra, Ghana (GH), Mumbai, India (MU), and Guam (GU). Temperate populations lie north of the Tropic of Cancer and were sampled in Vermont (VT), Indiana (IN), and North Carolina (NC). (**B**) Proportion of adult male flies responsive along a heat ramp (+1°C min^{-1} from 25°C). Tropical lines are indicated in solid pink.

Temperate lines are indicated in dotted blue. (**C**) Adult male CT_{max} was consistent across all populations (ANOVA, $F_{3,9} = 2.378$, $P = 0.1375$). CT_{max} extrapolated from the survival curves in B. Solid horizontal lines show means. Error bars indicate 95% confidence intervals. (**D**) Proportion of eggs successfully hatched following heat shock (45 min at indicated temperature). Tropical lines are indicated in solid pink. Temperate lines are indicated in dotted blue. (**E**) Embryonic thermal tolerance (LT_{50}) was higher in tropical lines than temperate lines (ANOVA, $F_{3,20} = 10.16$, $P = 0.0003$; Tukey's test, VT vs. IN, $q = 2.428$, $P = 0.3416$, VT vs. NC, $q = 0.4268$, $P = 0.9902$, IN vs. NC, $q = 2.666$, $P = 0.2656$, tropical vs. VT, $q = 6.909$, $P = 0.0005$, tropical vs. IN, $q = 4.04$, $P = 0.0444$, tropical vs. NC, $q = 4.04$, $P = 0.0005$). LT_{50} extrapolated from the survival curves in D. Solid horizontal lines show means. Error bars indicate 95% confidence intervals. *$P < 0.05$.

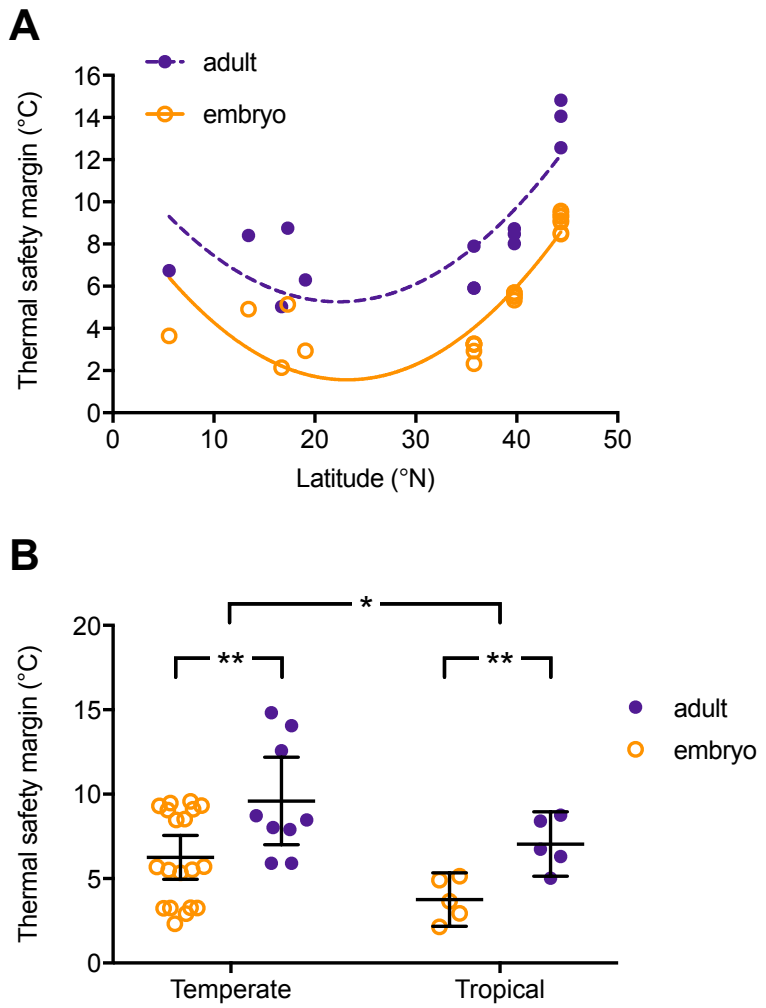


Figure 2.

Thermal safety margin differs by life stage and geographic region.

(**A**) Thermal safety margins were smaller for embryos than adults across latitude (Extra sum-of-squares F-test on fitness of two curves, $F_{3,32} = 15.95$, $P < 0.0001$; Non-linear least-squares regression, embryo, $R^2 = 0.8019$, $y = 0.01563x^2 - 0.7249x + 9.97$, adult, $R^2 = 0.6359$, $y = 0.01437x^2 - 0.6424x + 12.44$). Adults are indicated in purple filled circles with dashed purple regression line. Embryos are indicated in orange open circles with solid orange regression line. (**B**) Thermal safety margins were smaller for embryos than adults and smaller in the tropics than temperate sites (ANOVA, main effect of region, $F_{1,34} = 6.482$, $P = 0.0156$, main effect of life stage, $F_{1,34} = 11.15$, $P = 0.002$, region x life stage interaction, $F_{1,34} = 0.0006$, $P = 0.98$). Adults are indicated in purple filled circles, and embryos are indicated in orange open circles. Solid horizontal lines show means. Error bars indicate 95% confidence intervals. *$P < 0.05$, **$P < 0.01$.

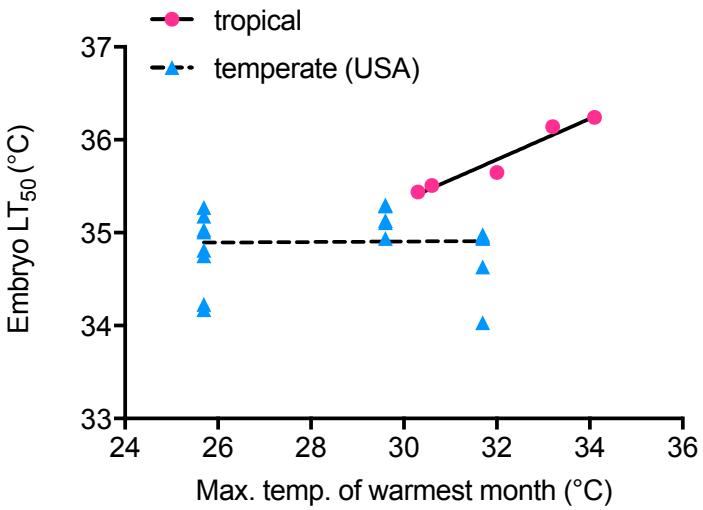
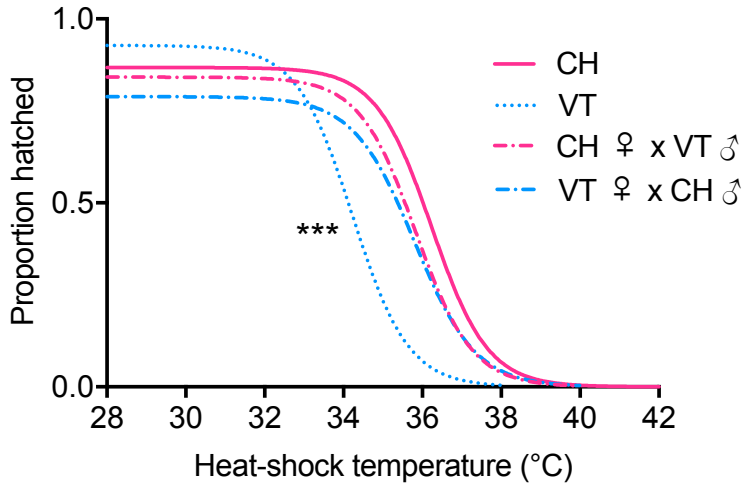


Figure 3.

Embryonic thermal tolerance and maximum habitat temperature (T_{max}) by region.

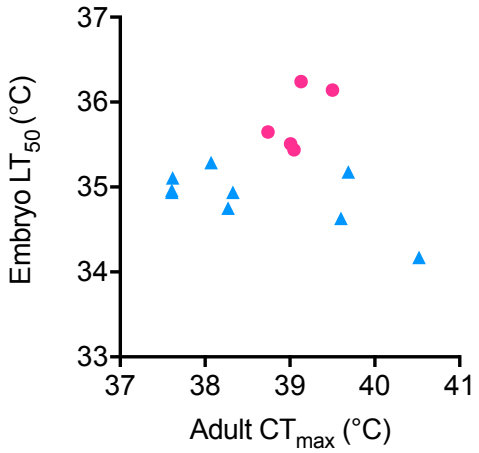
Embryonic thermal tolerance was positively correlated with T_{max} among tropical populations (Least-squares regression, $R^2 = 0.9478$, $P = 0.0051$, $y = 0.2199x + 28.75$) but not temperate populations (Least-squares regression, $R^2 = 0.0015$, $P = 0.9751$, $y = 0.00282x + 34.82$). Tropical genotypes are indicated in pink circles, with a solid black regression line fit. Temperate genotypes are indicated in blue triangles, with a dashed black regression line fit.



606
607 **Figure 4.**
608 **F1 progeny from tropical x temperate parents have high embryonic heat tolerance.**
609 Proportion of eggs successfully hatched following heat shock (45 min at indicated temperature)
610 among two parental genotypes that had the highest and lowest LT_{50} of all strains in this study,
611 CH (Chiapas, Mexico) and VT-12 (Vermont, USA), respectively, along with F1 progeny from
612 reciprocal crosses of these two parental lines, CH♀ x VT♂ and VT♀ x CH♂ (♀ = dam; ♂ = sire).
613 Note that VT-12 is labeled "VT" in the legend. LT_{50}: CH = 36.24°C, VT-12 = 34.23°C, CH♀ x
614 VT♂ = 35.83°C, VT♀ x CH♂ = 35.80°C (Logistic model, Extra sum-of-squares F-test on lower
615 LT_{50} of VT-12, $F_{3,166} = 6.695$, ***$P = 0.0003$).
616
617

Supplemental Table S1.
Stock information, collection locales, and thermal tolerance data for isofemale lines used in this study.

Stock No.	Locale	State/Country	Lat. (°N)	Long. (°E)	Adult CT_{max} (°C)	Embryo LT_{50} (°C)
VTECK_2	East Calais	Vermont, USA	44.3664	-72.4300	39.69	35.18
VTECK_4	East Calais	Vermont, USA	44.3664	-72.4300	-	35.03
VTECK_5	East Calais	Vermont, USA	44.3664	-72.4300	38.27	34.75
VTECK_8	East Calais	Vermont, USA	44.3664	-72.4300	-	34.81
VTECK_9	East Calais	Vermont, USA	44.3664	-72.4300	-	35.27
VTECK_10	East Calais	Vermont, USA	44.3664	-72.4300	40.52	34.17
VTECK_12	East Calais	Vermont, USA	44.3664	-72.4300	-	34.23
VTECK_14	East Calais	Vermont, USA	44.3664	-72.4300	-	35.01
BEA_5	Beasley Orchard	Indiana, USA	39.7637	-86.4766	-	35.13
BEA_16	Beasley Orchard	Indiana, USA	39.7637	-86.4766	37.62	35.11
BEA_21	Beasley Orchard	Indiana, USA	39.7637	-86.4766	38.33	34.94
BEA_32	Beasley Orchard	Indiana, USA	39.7637	-86.4766	-	35.30
BEA_36	Beasley Orchard	Indiana, USA	39.7637	-86.4766	38.07	35.29
RFM_4	Raleigh	North Carolina, USA	35.7636	-78.6627	-	34.98
RFM_6	Raleigh	North Carolina, USA	35.7636	-78.6627	-	34.95
RFM_16	Raleigh	North Carolina, USA	35.7636	-78.6627	-	34.03
RFM_19	Raleigh	North Carolina, USA	35.7636	-78.6627	39.60	34.63
RFM_34	Raleigh	North Carolina, USA	35.7636	-78.6627	37.61	34.96
RFM_48	Raleigh	North Carolina, USA	35.7636	-78.6627	37.61	34.94
14021-0231.22	Chiapa de Corzo	Chiapas, Mexico	16.7022	-93.0081	39.13	36.24
14021-0231.34	Monkey Hill	St. Kitts	17.3240	-62.7250	39.05	35.44
14021-0231.182	Accra	Ghana	5.5557	-0.1963	38.74	35.65
14021-0231.45	Mumbai	Maharashtra, India	19.0760	72.8777	39.50	36.14
14021-0231.198	Guam	Guam, USA	13.4443	144.7937	39.01	35.51

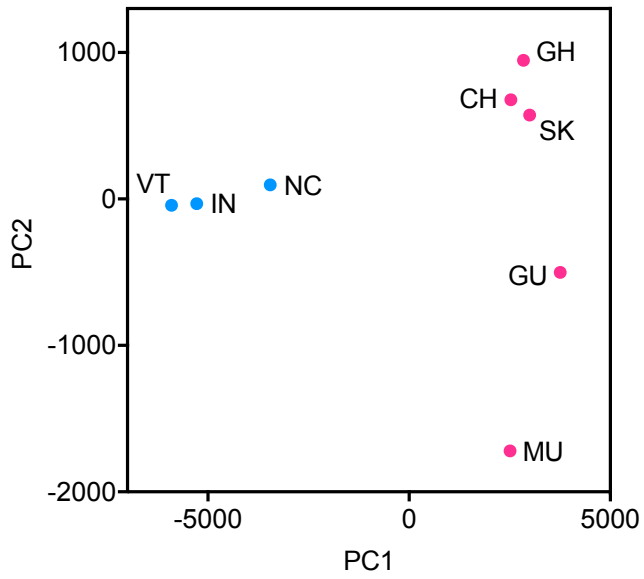


Supplemental Figure S1.

No significant relationship between adult CT_{max} and embryo LT_{50}.

Adult thermal tolerance and embryonic thermal tolerance were not correlated among all isofemale lines (Least-squares linear regression, $R^2 = 0.0005$). Tropical isofemale lines are shown in pink circles and temperate isofemale lines are shown in blue triangles.

630



631

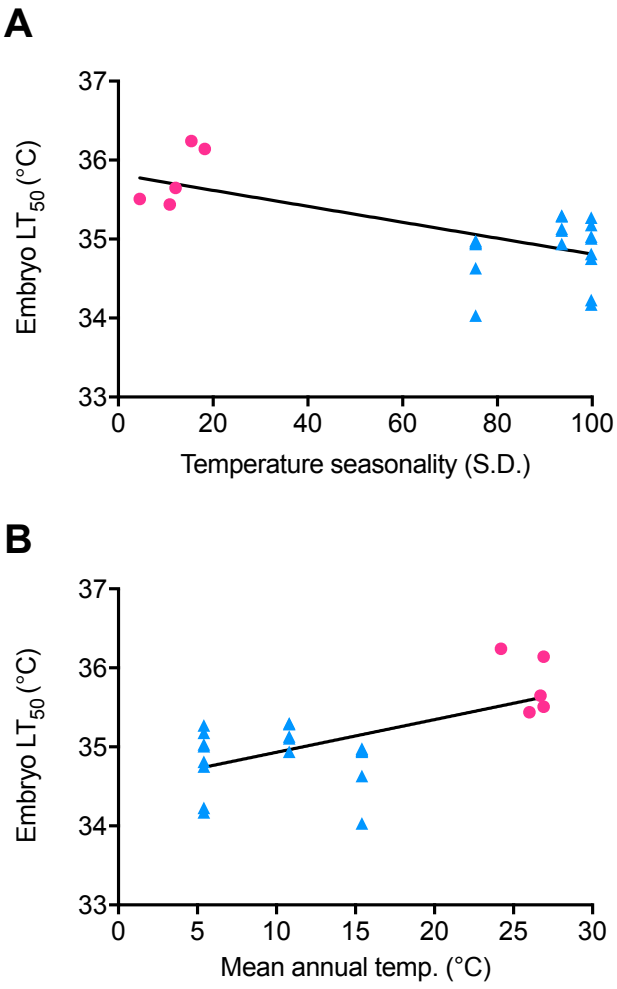
632 **Supplemental Figure S2.**

633 **Principal components analysis of 19 Bioclim variables at each of the 8 collection sites.**

634 Plotted are scores of the first two principle components (PC1 vs. PC2), which describe the major
635 axes of variation in 19 bioclimatic variables among the collection sites. PC1 and PC2 explained
636 95.6% and 4%, respectively, of the variation in climate among sites. Temperature seasonality
637 was the top component loading on PC1, explaining 98% of the variation in PC1, and annual
638 precipitation was the top component loading on PC2, explaining 49% of the variation in PC2.
639 Temperate sites are shown in blue and tropical sites in pink.

640

641

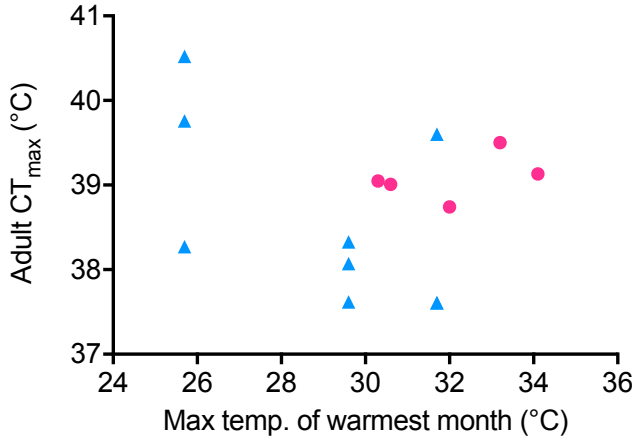


Supplemental Figure S3.

Relationship between embryonic thermal tolerance and (A) temperature seasonality and (B) mean annual temperature.

(**A**) Temperature seasonality is the standard deviation in temperature (°C) over the course of the year, averaged across the years 1960 to 1990 for each of the sites. Solid black line represents the regression line fit, which had a significantly negative slope (Least-squares linear regression, R^2 = 0.42, P = 0.0006, y = -0.01x + 35.82). (**B**) Mean annual temperature is the average yearly temperature from 1960 to 1990 for each of the sites. Solid black line represents the regression line fit, which had a significantly positive slope (Least-squares linear regression, R^2 = 0.37, P = 0.0016, y = 0.041x + 34.52). In both A and B, tropical isofemale lines are shown in pink circles and temperate isofemale lines are shown in blue triangles.

655



656

657 **Supplemental Figure S4.**

658 **No significant relationship between adult thermal tolerance (CT_{max}) and maximum habitat**
659 **temperature (T_{max}).**

660 Variation in adult thermal tolerance showed no correspondence to variation in T_{max} among all
661 collection sites (Least-squares linear regression, $R^2 = 0.055$). Tropical isofemale lines are shown
662 in pink circles and temperate isofemale lines are shown in blue triangles.