Title: Integrating patterns of thermal tolerance and phenotypic plasticity with population genetics to improve understanding of vulnerability to warming in a widespread copepod

Running Title: Factors affecting vulnerability to warming

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Abstract:

Differences in population vulnerability to warming are defined by spatial patterns in thermal adaptation. These patterns may be driven by natural selection over spatial environmental gradients, but can also be shaped by gene flow, especially in marine taxa with high dispersal potential. Understanding and predicting organismal responses to warming requires disentangling the opposing effects of selection and gene flow. We begin by documenting genetic divergence of thermal tolerance and developmental phenotypic plasticity. Ten populations of the widespread copepod *Acartia tonsa* were collected from sites across a large thermal gradient, ranging from the Florida Keys to Northern New Brunswick, Canada (spanning over 20 degrees latitude). Thermal performance curves from common garden experiments revealed local adaptation at the sampling range extremes, with thermal tolerance increasing at low latitudes and decreasing at high latitudes. The opposite pattern was observed in phenotypic plasticity, which was strongest at high latitudes. Over a large portion of the sampled range, however, we observed a remarkable lack of differentiation of thermal performance curves. To examine whether this lack of divergence is the result of selection for a generalist performance curve or constraint by gene flow, we analyzed cytochrome oxidase I mtDNA sequences, which revealed abundant genetic

diversity and widely-distributed haplotypes. Strong divergence in thermal performance within genetic clades, however, suggests that the pace of thermal adaptation can be relatively rapid. The combined insight from the laboratory physiological experiments and genetic data indicate that gene flow constrains differentiation of thermal performance curves. This balance between gene flow and selection has implications for patterns of vulnerability to warming. Taking both genetic differentiation and phenotypic plasticity into account, our results suggest that local adaptation does not increase vulnerability to warming, and that low latitude populations in general may be more vulnerable to predicted temperature change over the next century.

Keywords:

Climate change, Climate Variability Hypothesis, Copepod, Gene flow**,** Local adaptation, Macrophysiology, Phenotypic plasticity, Plankton, Thermal performance, Rapid adaptation

Introduction:

Temperature affects processes at every level of biological organization (Angilletta, 2009; Hochachka & Somero, 2002). The rapid warming of the world's oceans (Cheng *et al.,* 2019a; Cheng *et al.,* 2019b; Roemmich *et al.,* 2015; Saba *et al.,* 2016; Wijffels *et al.,* 2016) presents a significant threat to contemporary marine biodiversity (Bryndum-Buchholz *et al.,* 2019; Parmesan, 2006). In addition to the increase in mean ocean temperature, significant increases in the magnitude and frequency of acute events like heat waves have been predicted (Lorenzo & Mantua, 2016; Meehl, 2004; Perkins *et al.,* 2012). These acute events have significant consequences for organisms, and therefore cannot be ignored in predictions of biotic response to climate change (Campbell-Staton *et al.,* 2017; Leicht *et al.,* 2017; Stoks *et al.,* 2017; Sydeman *et al.,* 2013; Ummenhofer & Meehl, 2017; Wernberg *et al.,* 2016). Vulnerability to these climatic changes is established by pre-existing spatial patterns in thermal adaptation. Characterizing

patterns of thermal adaptation and determining their underlying causes is, therefore, directly related to our ability to predict vulnerability and responses of the biota to climate change (Moran *et al.,* 2016; Sorte *et al.,* 2011).

Macrophysiology, the study of variation in physiological traits across space and time (Chown *et al.,* 2004), often yields evidence for adaptation across environmental gradients. Latitudinal thermal gradients, for example, are well-known drivers of local adaptation of thermal tolerance (Addo-Bediako *et al.,* 2000; Castañeda *et al.,* 2015; Gaitán-Espitia *et al.,* 2017; Pereira *et al.,* 2017; Yampolsky *et al.,* 2013). These patterns in adaptation across large spatial scales are often attributed to selection acting on a set of populations, as gene flow is assumed to be relatively low over large distances. This important assumption may not always hold for marine taxa which often have high dispersal potentials (Bowen *et al.,* 2016; Carlton *et al.,* 2017; Cowen *et al.,* 2006; Cowen & Sponaugle, 2009; Gélin *et al.,* 2017; Kinlan & Gaines, 2003; Sexton & Norris, 2008). However, dispersal dynamics in the ocean can be complex (McManus & Woodson, 2012), preventing easy generalization or prediction of connectivity. Instead, genetic markers are often required to estimate levels of connectivity between populations (Palumbi, 2003).

Adaptive genetic differentiation and phenotypic plasticity are two of the main mechanisms used by organisms to cope with variation in the thermal environment. (Angilletta, 2009; Dam, 2013; Magozzi & Calosi, 2014; Somero, 2010; Sparks *et al.,* 2017). Adaptive genetic differentiation is well-known to produce significant variation in phenotypes (Hochachka & Somero, 2002). Phenotypic plasticity, the capacity of a single genotype to produce multiple phenotypes in response to different environmental conditions, can also have large effects (Ayrinhac *et al.,* 2004; Chown *et al.,* 2004, West-Eberhard 2003). Several types of phenotypic plasticity, including acclimation (Stillman, 2003), hardening (Sørensen *et al.,* 2001), and developmental

70 phenotypic plasticity (Pereira *et al.,* 2017), have all been shown to have strong effects on
71 organismal thermal tolerance. Both mechanisms are likely to play important roles in determining
72 organismal responses to climate change (Hoffmann & Sgro, 2011; Reusch, 2013). Importantly,
73 plasticity acts within generations, and might therefore provide a mechanism for rapid response to
74 environmental variability (Chevin *et al.,* 2010; Chown *et al.,* 2007; Merilä & Hendry, 2014;
75 Seebacher & Grigaltchik, 2014). Plasticity may also prevent the loss of cryptic genetic diversity
76 by shielding genotypes from selection (Friedrich & Meyer, 2016; Pfennig *et al.,* 2010;
77 Schlichting, 2004). This is in stark contrast to selection on standing genetic diversity, which may
78 result in strong demographic bottlenecks and the loss of genetic diversity (Corbett-Detig *et al.,*
79 2015; Hoffmann & Sgrò, 2011; Kellermann *et al.,* 2009). Studies that examine both mechanisms
80 across large spatial scales are needed.

81 Characterizing the spatial patterning of phenotypic plasticity and adaptive genetic differentiation
82 is important for predictions of population vulnerability to warming. The Climate Variability
83 Hypothesis (CVH) (Janzen, 1967; Stevens, 1989) posits that thermal tolerance should correspond
84 to the mean temperature experienced by a population whereas phenotypic plasticity should
85 evolve in response to variability in the thermal environment. This hypothesis has accumulated
86 support over time, especially in terrestrial and freshwater systems (Deutsch *et al.,* 2008; Sunday
87 *et al.,* 2010), but still lacks robust experimental validation in the marine realm. It has also been
88 proposed that patterns in the evolution of phenotypic plasticity may be the result of a trade-off
89 between thermal tolerance and the strength of phenotypic plasticity (Stillman, 2003), where
90 higher thermal tolerance evolves at the expense of the capability to modify the phenotype via
91 plasticity.

Patterns in adaptation can, however, also be strongly influenced by gene flow (Lenormand, 2002). The "Gene Flow vs. Selection" issue has been at the heart of evolutionary ecology for decades (Blanquert *et al.* 2013; Slatkin, 1985; 1987). Successful gene flow between populations can strongly impede local adaptation (Garant *et al.,* 2007; Hendry & Taylor, 2004; Lenormand, 2002; Moore *et al.,* 2007; Nosil & Crespi, 2004), and phenotypic divergence is often correlated with the degree of isolation (Mayr, 1963). However, low levels of gene flow might also promote local adaptation by increasing the genetic diversity contained within a population (Garant *et al.,* 2007; Tallmon *et al.,* 2004). The potential for interaction between selection and gene flow makes integrated approaches to studying evolutionary physiology critical for robust characterization of spatial patterns in adaptation. Taking both selection and gene flow into account may be needed to explain observed patterns of adaptation (Dionne *et al.,* 2008; Moore & Hendry 2005).

Spatial patterns in both adaptation and predicted warming interact to produce what is likely to be spatially heterogeneous vulnerability to warming. Understanding which populations are more vulnerable to warming, and why, is critical for effective management and conservation of diversity. Previous work has suggested that warm-adapted, low latitude species or populations are more vulnerable to climate change as they already experiencing temperatures near their thermal maxima (Comte & Olden, 2017; Tewksbury *et al.*, 2008; Vinagre *et al.*, 2016). However, this is not a universal observation, and populations from mid- to high-latitudes have also been predicted to be more vulnerable (Bennett *et al.*, 2015; Calosi *et al.*, 2008; Fusi *et al.*, 2015). Additionally, these predictions are often based on measurements of thermal tolerance. This is insufficient, however, as phenotypic plasticity may also play a large role in determining vulnerability to climate change (Burggren, 2018; Chown *et al.*, 2007; Magozzi & Calosi, 2015; Sparks *et al.*, 2017). Examining spatial patterns in both thermal tolerance and the strength of phenotypic plasticity may provide more robust estimates of vulnerability.

Copepods are the most abundant metazoans in the ocean (Humes, 1994; Turner, 2004). They play an important role in transferring energy from primary producers to secondary consumers and are therefore tightly linked to global biogeochemical systems (Menden-Deuer & Kiørboe, 2016), marine trophic webs (Turner, 2004), and commercial fisheries (Castonguay *et al.,* 2008; Dam and Baumann, 2017; Friedland *et al.,* 2012). Studies of thermal adaptation in copepods have a long history (Bradley, 1978; Lonsdale & Levinton, 1985), but local adaptation has largely been ignored, especially in pelagic species (but see Smolina *et al.,* 2016). The calanoid copepod *Acartia tonsa* often dominates coastal and estuarine environments. With a large latitudinal distribution (Turner, 1981) and a life-history amenable to laboratory culturing, this is an ideal model species for studying spatial patterns in adaptation. Previous studies of *Acartia* species have observed local adaptation to several different factors, including exposure to toxic dinoflagellates (Colin & Dam, 2002; 2004), salinity (Plough *et al.,* 2018), and pH (Aguilera *et al.,* 2016). Limited evidence also exists that local adaptation to temperature occurs across large geographic scales (González, 1974; Sasaki *et al.,* 2019). This past work suggests that low latitude populations may be more vulnerable to warming (Sasaki *et al.*, 2019), but conclusions are limited by the lack of spatial coverage and the number of populations used. Additionally, no population genetic information was included. Population genetic studies have shown genetic structuring in this taxon to be complex, with abundant cryptic diversity (Caudill & Bucklin, 2004; Chen & Hare, 2011). The effects of this genetic diversity on patterns of adaptation has largely been ignored (but see Plough *et al.* 2018). Here we examine both large- and fine-scale spatial patterns in the effects of genetic differentiation and phenotypic plasticity on the thermal performance curves (TPCs) of *Acartia tonsa*. We observe clear latitudinal variation in both thermal tolerance and the magnitude of the plastic response, but also find a remarkable lack of divergence over a large portion of the sampled range. Paired with insights from the analysis of

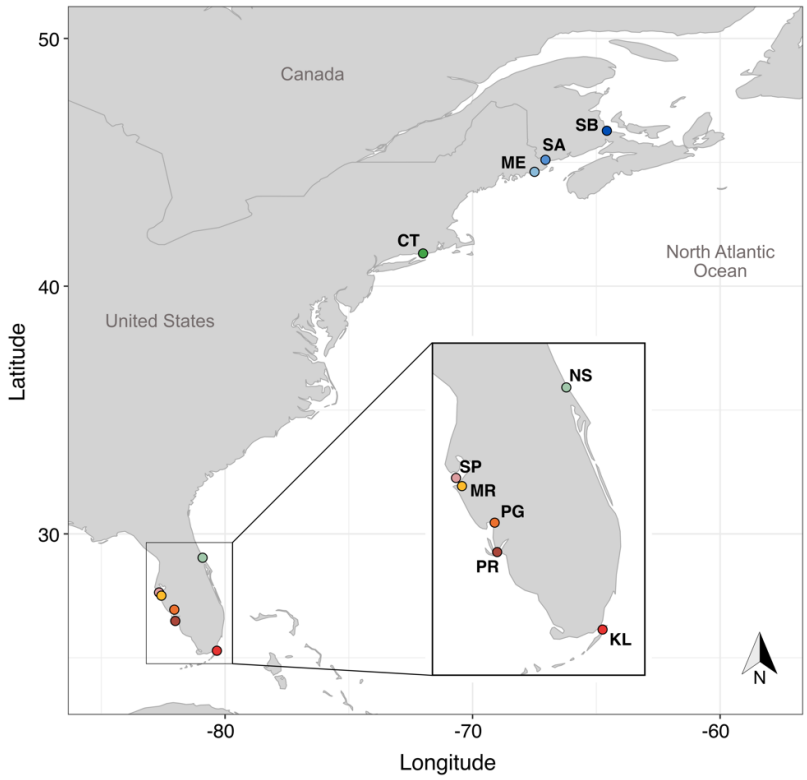
cytochrome oxidase I (COI) mtDNA sequence data, we suggest that this lack of divergence is due to constraint by gene flow rather than selection for a generalist performance curve. These spatial patterns in adaptation will likely affect vulnerability to warming, with southern populations being the most vulnerable.

Methods:

Sampling and Culture Maintenance

Copepods were collected from ten sites spanning 21 degrees of latitude, ranging from the Florida Keys to the Northumberland Strait in Canada (Fig. 1; Table 1). Sites selected cover a range of thermal environments, with mean monthly temperatures ranging from 9.05 – 25.1°C, and average monthly temperature ranges varying between 1.38 – 12.88°C. At each site, samples were collected in surface plankton tows using a 250-µm mesh plankton net with a solid cod end. Water column depth was less than 10 m at all sites. Salinity and surface water temperature were recorded at the time of collection. Within 3 hours of collection, mature *Acartia tonsa* individuals were visually identified using a dissection microscope and sorted into 0.6-µm filtered sea water (FSW), with salinity and temperature adjusted to match collection conditions. Each culture began with more than 1000 mature females and abundant males to ensure fertilization. Additional individuals were preserved in 95% molecular grade ethanol for later genetic analysis. All samples and cultures were then transported by car back to the University of Connecticut, Avery Point campus. Copepods were transported in temperature-controlled containers with particular care to maintain temperature and salinity near collection conditions. Aquarium bubblers were used to keep containers well oxygenated. Copepods were fed with a mixture of a green flagellate (*Tetraselmis* sp.) and a small diatom (*Thalassiosira weissflogii*) during transport. In the laboratory, live cultures were gradually brought to 18°C and 30 practical salinity units (psu) and

163 then maintained for several generations under constant conditions to minimize the effects of
164 previous environmental acclimation. Aquarium bubblers were used to ensure cultures were well
165 oxygenated. The water of each culture was changed weekly. During this period, cultures were
166 fed *ad libitum* a mixture of a green flagellate (*Tetraselmis* sp.), a small diatom (*Thalassiosira*
167 *weissflogii*), and a cryptomonad (*Rhodomonas salina*). Phytoplankton were cultured semi-
168 continuously in F/2 medium (without silica for *Tetraselmis* and *Rhodomonas*) with a 12 hr:12 hr
169 light:dark cycle at 18°C. Genetic samples were kept at -80°C until extraction.



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171 Figure 1: Map of sampling locations in the North Atlantic and Gulf of Mexico. The inset is a
172 closer view of the southern sampling sites, to better show the spatial arrangement. Sampled site
173 names are abbreviated: Shediac Bay (SB), St. Andrew (SA), Maine (ME), Connecticut (CT),
174 New Smyrna (NS), St. Petersburg (SP), Manatee River (MR), Punta Gorda (PG), Punta Rasa
175 (PR), and Key Largo (KL). Additional site details can be found in Table 1.

176 Table 1: Collection site details for each of the ten populations recovered. Site names are
177 provided, along with the two-letter abbreviation used. Water body refers to the general area
178 where each site is located. The latitude of each site is provided, along with the salinity of
179 collection. High salinity refers to sites with a salinity greater than 28 psu, while the low salinity
180 sites both had a salinity of 5 psu. Climatological temperature data for each of the sites are also
181 included. Raw temperature data was acquired from the MODIS-aqua sea surface temperature
182 database and summarized to monthly intervals using CDO (Schulzweida *et al.,* 2009).

Population	Abbr.	Water Body	Latitude	Salinity	Monthly Mean Temp. (°C)	Monthly Maximum Temp. (°C)	Monthly Minimum Temp. (°C)	Monthly Temperature Range (°C)	Monthly Temperature Variance (°C)
Key Largo	KL	Florida - Atlantic	25.2838	High	25.10	29.56	23.73	2.87	1.91
Punta Rasa	PR	Florida - Gulf Coast	26.4835	High	24.79	29.42	19.35	10.47	8.17
Punta Gorda	PG	Florida - Gulf Coast	26.9403	Low	24.69	28.80	20.04	9.05	7.06
Manatee River	MR	Florida - Gulf Coast	27.5056	Low	20.77	21.84	19.87	1.99	1.79
St. Petersburg	SP	Florida - Gulf Coast	27.5073	High	24.02	29.50	17.66	1.38	9.59
New Smyrna	NS	Florida - Atlantic	29.0374	High	22.84	26.71	18.60	8.45	6.35
Connecticut	CT	Long Island Sound	41.3205	High	12.52	18.69	6.55	12.88	17.41
Machias	MA	Gulf of Maine	44.6150	High	9.05	11.87	6.41	5.81	5.77
St. Andrew	SA	Bay of Fundy	45.1000	High	9.37	13.06	5.75	7.72	9.81
Shediac Bay	SB	Northumberland Strait	46.272	High	12.38	15.76	8.31	12.06	19.66

183

184 Heat Stresses and Performance Curve Estimation

185 After two generations, a split-brood common garden experiment was used to examine the effects
186 of genetic differentiation and developmental phenotypic plasticity on the thermal performance
187 curve (TPC) of *Acartia tonsa*. For each population, eggs were collected and randomly split
188 between two developmental temperatures, 18ºC and 22ºC. All other conditions were kept the
189 same between treatments. Upon reaching maturity, females were collected from both
190 developmental conditions and exposed to a 24-hour acute heat stress. Healthy individuals were
191 placed into 1.5 mL of FSW in a 2.0 mL microfuge tube, which was then partially capped to
192 allow for gas exchange with the atmosphere. Only one female was placed into each tube. After
193 all tubes were filled, they were left to rest for one hour at their respective developmental
194 temperatures. Tubes were then placed into dry baths and exposed to a single temperature,
195 ranging from 18ºC to 38ºC. The number of females per temperature varied, with fewer
196 individuals exposed to the lowest and highest temperatures (where survivorship was expected to
197 be least variable) and more individuals at intermediate temperatures. At least six females were
198 used per temperature. Each female experienced only one temperature throughout the duration of
199 the assay and was used for only one assay. After 24 hours, tubes were collected, and survivorship
200 was determined visually using a dissection scope. Copepods were marked as alive if there was an
201 active response to external stimuli or visible gut passage movement. Evaporation (and therefore
202 fluctuations in salinity) was negligible during the heat stress.

203 All statistical analyses were performed using the software package R (R Core Team, 2016).
204 Logistic regressions were used to estimate TPCs for the two developmental treatments for all ten
205 populations. A three-way ANOVA was used to examine the effects of genetic differentiation and
206 phenotypic plasticity on the TPCs (survival as a function of stress temperature, population, and

developmental temperature). An effect of genetic differentiation would be indicated by a significant population term, while an effect of developmental phenotypic plasticity would be indicated by a significant developmental temperature term. Population differences in the strength of phenotypic plasticity would be indicated by a significant population x developmental temperature term (a heterogeneity of slopes test).

Each curve was also summarized by estimation of LD_{50} values (the temperature at which 50% survivorship would be observed). This is a common metric for thermal tolerance. We define the difference in LD_{50} values between the two developmental treatments (ΔLD_{50}) as the strength of developmental phenotypic plasticity. Standard error for ΔLD_{50} values were calculated as $\sqrt{(SE_{18C}^2 + SE_{22C}^2)}$, where SE_{18C} and SE_{22C} are the standard error estimates for LD_{50} from the 18ºC and 22ºC developmental temperature groups, respectively. The effect of plasticity was also estimated by calculating the average increase at all survivorship levels from 10% - 90%, which we call $\Delta LD_{average}$. This takes into account any potential population-specific non-linearities in the effect of phenotypic plasticity across the TPC. We also estimated LD_{10} (temperature of 10% survival) and ΔLD_{10} values as estimates of thermal limits.

Climatologies and Correlations

Daily satellite sea-surface temperature (SST) measurements for the years 2000 - 2017 were retrieved for each geographic location from the MODIS-Aqua database (https://oceancolor.gsfc.nasa.gov/data/aqua/). Because *A. tonsa* has generation times on the order of weeks to a month, this data was summarized as average monthly climatological parameters (mean, maximum, and minimum temperatures as well as temperature range and variance; Table 1) using the command line Climate Data Operators (CDO; Schulzweida *et al.,* 2009).

The CVH postulates that thermal tolerance (LD_{50}) should respond to some mean representation of the thermal environment, while the strength of the phenotypic plasticity (ΔLD_{50}) should reflect the variation in the thermal environment. To test these predictions, we regressed our metrics of thermal adaptation against the environmental parameters of interest (LD_{50} ~ developmental temperature + monthly mean + monthly maximum + monthly minimum; ΔLD_{50} ~ monthly temperature variance + monthly temperature range). A linear regression was also used to examine the relationship between thermal tolerance and the strength of plasticity. Trade-offs between thermal tolerance and the strength of phenotypic plastic (Stillman, 2003) would be suggested by a significant negative relationship between LD_{50} and ΔLD_{50}. Correlations between the environmental parameters and latitude, as well as with each other were also examined.

Vulnerability to Warming

The statistical model used to estimate TPCs from the common garden experiment can also be used to predict what would happen if parameters like developmental temperature and stress temperature change. We use this to estimate vulnerability to warming across the ten populations examined. For each population, we estimated the TPC with the mean monthly temperature as the developmental temperature, using the *predict.glm* function in R (*stats* package). Survivorship at the mean monthly maximum temperature was then taken from this modelled TPC. The magnitude of predicted warming at each site over the next century was visually estimated from a high resolution model of warming in the North Atlantic (Saba *et al.* 2016). Future survivorship was estimated in a similar manner using these future temperature values (Supp. Table 1). Vulnerability was estimated as the difference between current and future survivorship values; a positive difference indicates an increase in survivorship (less vulnerable to warming) while a negative value indicates a decrease in survivorship (more vulnerable to warming).

252 DNA Extraction, Amplification, and Sequencing

253 Mitochondrial cytochrome-oxidase I (COI) sequence data was generated for individuals from all
254 ten populations (n = 17 - 34 per population). DNA was extracted using a Qiagen Blood and
255 Tissue kit following the manufacturer's instructions. Extracted DNA was eluted in 50 µl of
256 elution buffer (25 µl twice) and stored at -20°C. COI sequences were amplified by polymerase
257 chain reaction (PCR) using mtCOI primers LCO1490 (forward:
258 GGTCAACAAATCATAAAGATATTGG) and HCO2198 (reverse:
259 TAAACTTCAGGGTGACCAAAAAATCA) (Folmer *et al.,* 1994). All PCR reactions were
260 performed in 24 µl volumes with 13 µl ExTaq HS polymerase (Takara Bio Inc.), 1 µL each
261 forward and reverse primers, 5 µl genomic DNA, and 5 µl ultrapure molecular grade water. The
262 optimized PCR protocol began with an initial denaturation of 94°C for 3 minutes followed by 35
263 cycles of denaturation at 94°C for 45 seconds, annealing at 48°C for 45 seconds, and extension at
264 72°C for 45 seconds. The protocol ended with a final extension at 72°C for 7 minutes.
265 Amplification success and product length were confirmed visually using a 1.2% agarose gel
266 post-stained with GelRed (Biotium Inc.). Successful amplification products were then purified
267 using an ExoSAP-IT PCR clean-up kit (ThermoFisher Scientific) following manufacturer's
268 instructions before being sent to Eurofins Genomics for forward and reverse strand sequencing.

269 Sequence Analysis

270 Consensus sequences were generated for each individual using forward and reverse strands in
271 UniPro (Okonechnikov *et al.,* 2012). Sequences were aligned using Clustal-W (Thompson *et al.,*
272 1994) and then visually checked. Species identity of each sequence was verified by BLAST
273 search in NCBI's GenBank database (Sayers *et al.,* 2018). Population genetic summary statistics
274 (nucleotide diversity (π), haplotype diversity (Hd), and average number of nucleotide differences

between haplotypes) were calculated using DNaSP v6 (Librado & Rozas, 2009). A haplotype network using an infinite site model was computed using the R package *Pegas* (Paradis, 2010). MigrateN was used to estimate mutation-scaled population size and migration rate values (Beerli, 2006; Beerli & Felsenstein, 2001). The transition-transversion ratio was set to 20. A uniform migration rate prior was set with a minimum of 0 and a maximum of 2000, while the population size prior was set with a minimum of 0.001 and a maximum of 0.1. Analyses entailed a single long MCMC chain with 4 concurrent replicates and a static heating scheme. 10,000,000 steps were recorded with a burn-in of 500,000. All other settings used default values. The number of migrants per generation (N_M) was then calculated as $N_{M(pop1->pop2)} = \Theta_{pop1} * M_{pop1->pop2}$. A linear regression between the number of migrants per generation and the pairwise population difference in LD_{50} was used to investigate constraint of thermal adaptation by gene flow.

Results:

Differentiation of Thermal Performance Curves

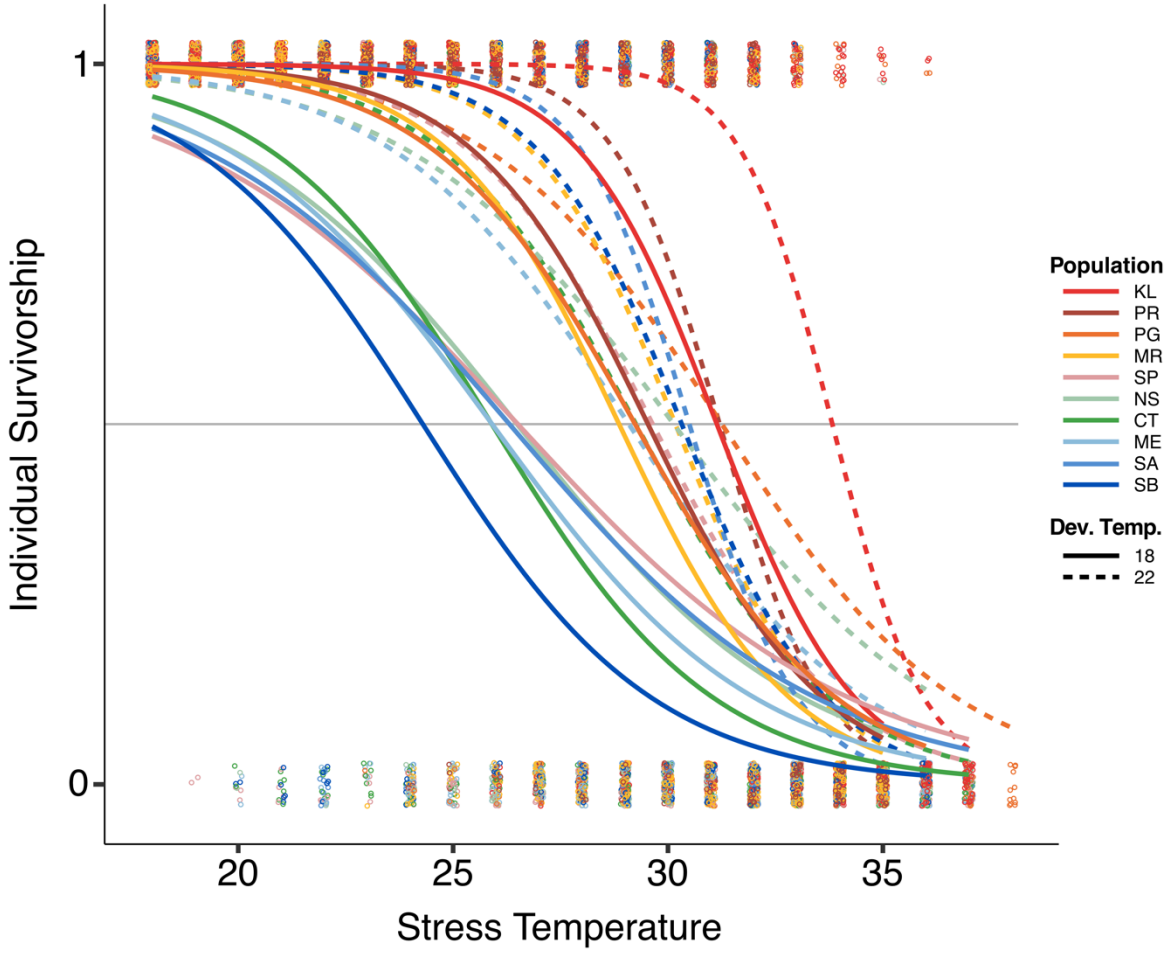
A total of 6144 females were used to estimate the thermal performance curves (Fig. 2). The ANOVA results for the TPCs yield a significant effect of both population and developmental temperature (both $p < 2.2x10^{-16}$; Table 2), indicating an influence of both genetic differentiation and developmental phenotypic plasticity, respectively. The significant interaction term between stress temperature and population ($p < 2.2x10^{-16}$) indicates that genetic differentiation results in changes to the shape of the performance curve, while the significant interaction term between population and developmental temperature ($p = 1.45x10^{-7}$) indicates that the strength of phenotypic plasticity differs between populations.

297 Table 2: Results of an ANOVA on the logistic regression of survivorship against stress
298 temperature, population, and developmental temperature. All terms are statistically significant (*p*
299 values << 0.0002)

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)
Stress Temp	1	3024.26	6140	5158.1	< 2.2e-16
Population	9	351.38	6131	4806.8	< 2.2e-16
Dev Temp	1	266.91	6130	4539.8	< 2.2e-16
Stress Temp * Pop	9	102.14	6121	4437.7	< 2.2e-16
Stress Temp * Dev Temp	1	23.95	6120	4413.8	9.90E-07
Pop * Dev Temp	9	49.31	6111	4364.4	1.45E-07
Stress Temp * Pop * Dev Temp	9	32.45	6102	4332	0.0001665

300

301 These results are clearly evident in the TPCs (Fig. 2). Shediac Bay, the northernmost population,
302 has a TPC shifted towards cooler temperatures while the southernmost population, Key Largo,
303 has a TPC shifted towards warmer temperatures. Two groups of intermediate TPCs are also
304 observed, one containing the populations from Manatee River, Punta Gorda, and Punta Rasa,
305 with the other comprised of all remaining populations. This pattern can also be seen in the LD_{50}
306 values of the 18°C developmental temperature group (Fig. 3). Differences are reduced in the
307 22°C developmental temperature group.



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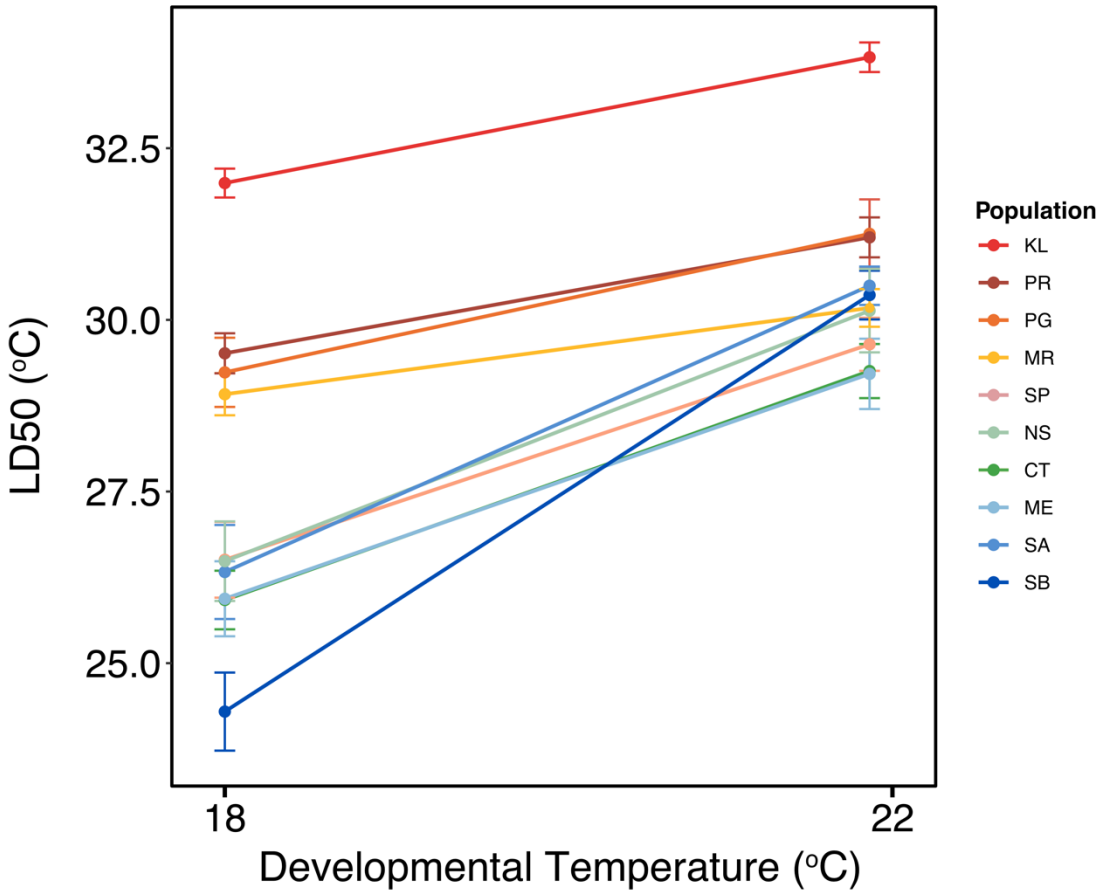
309 Figure 2: Thermal survivorship curves for each of the ten populations (different colors) and
310 developmental temperatures (solid vs. dashed lines). Survivorship was measured using
311 individuals from a split-brood, common garden experiment and a 24-hour acute heat shock.
312 Survivorship was recorded as binary data (1 = survived, 0 = died), with curves estimated by
313 logistic regression.



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315 Figure 3: Reaction norms of thermal tolerance (LD_{50}) for the ten populations, shown in different
316 colors. LD_{50} was calculated as the temperature at which 50% survivorship was observed in the
317 thermal performance curve. Error bars show standard errors. The slope of the individual norms
318 represents the strength of developmental phenotypic plasticity (ΔLD_{50}).

319 Thermal tolerance increased with warmer developmental temperature in all populations,
320 suggesting a ubiquitous effect of developmental phenotypic plasticity. Shediac Bay, the
321 northernmost population had the largest strength of phenotypic plasticity while Manatee River, a
322 population from the Gulf Coast of Florida, had the smallest. The various crossed reaction norms
323 also indicate variation in the strength of plasticity. The effects of developmental phenotypic

plasticity were stronger for thermal tolerance than thermal limits ($\Delta LD_{50} > \Delta LD_{10}$; Supp. Fig. 1). There are no large differences between ΔLD_{50} and $\Delta LD_{average}$ (Supp. Fig. 2), so we will focus only on ΔLD_{50} for the sake of uniformity with the LD_{50} metric of thermal tolerance.

Lack of Differentiation in Thermal Performance Curves

Interestingly, we also observed a striking lack of differentiation in the TPCs of populations from a large portion of the sampling range, indicated by the boxes in Fig. 4. Thermal tolerance values for the St. Petersburg (SP), New Smyrna (NS), Connecticut (CT), Maine (ME), and Saint Andrew (SA) populations, spanning over 20 degrees latitude and originating from drastically different thermal environments, were remarkably similar. This lack of differentiation is also seen in the strength of developmental phenotypic plasticity.

Environmental Correlations

Despite the lack of differentiation between some populations, LD_{50} and ΔLD_{50} were both significantly correlated with latitude ($p = 0.0017$ and 0.008 respectively; Table 3; Fig. 4). ANOVA results show LD_{50} to be significantly correlated with both mean monthly temperature ($p = 3.7 \times 10^{-4}$) and mean monthly minimum temperature ($p = 0.0012$; Table 4; Fig. 5). ΔLD_{50} is significantly correlated with mean monthly temperature variance ($p = 0.015$) but not mean monthly temperature range ($p = 0.58$; Table 4; Fig. 5). Both mean monthly temperature and mean monthly temperature variance were significantly correlated with latitude ($p = 1.81 \times 10^{-6}$ and 0.045 respectively; Supp. Fig. 3, 4) but not with each other ($p = 0.14$; Supp. Fig. 5). There is a significant correlation between LD_{50} and ΔLD_{50} ($p = 0.0023$; Fig. 6).

344 Table 3: ANOVA results for the correlation between thermal tolerance and the strength of

345 phenotypic plasticity with latitude.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
LD_{50}					
Dev. Temp.	1	45.923	45.923	23.3632	0.0001834
Latitude	1	27.812	27.812	14.1493	0.0017056
Dev. Temp. x Latitude	1	5.4	5.4	2.7474	0.1168863
Residuals	16	31.45	1.966		
ΔLD_{50}					
Latitude	1	11.1685	11.1685	12.196	0.008171
Residuals	8	7.3261	0.9158		

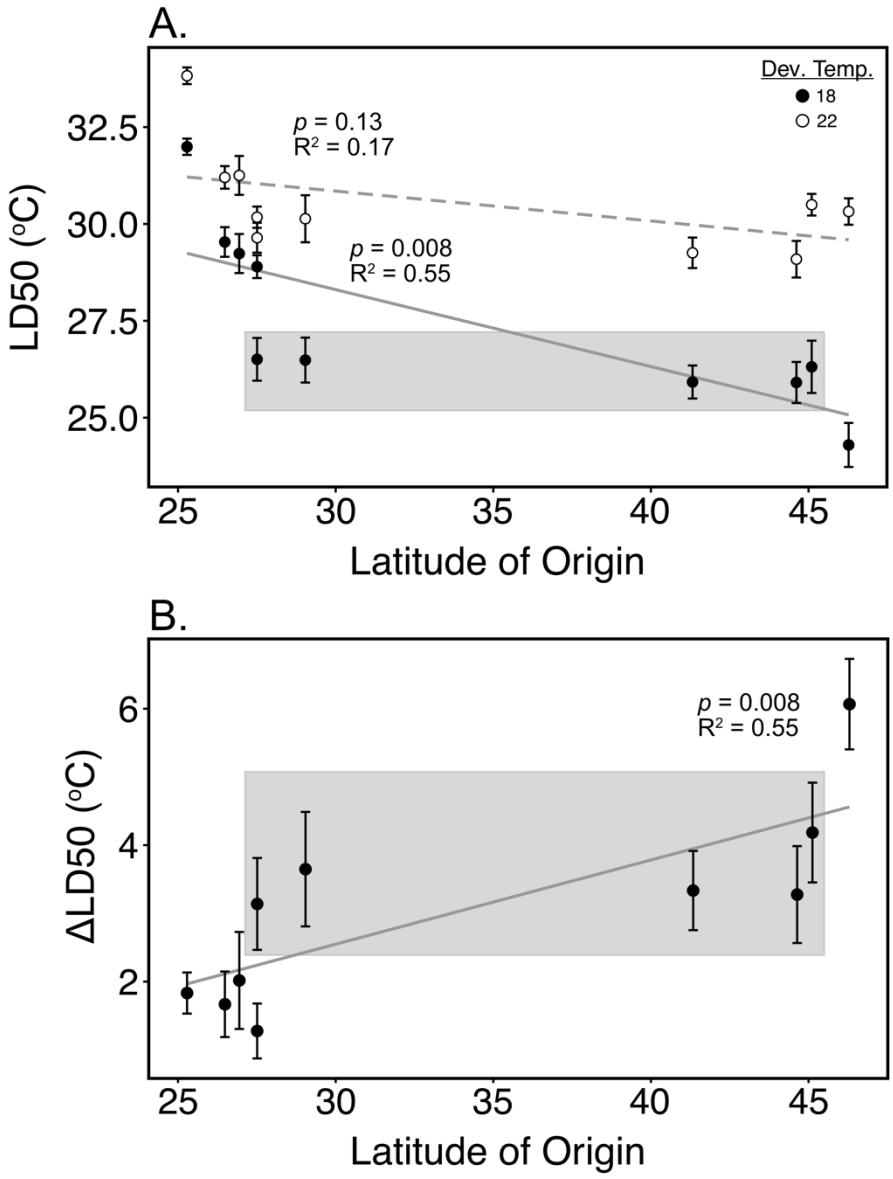
346

347 Table 4: ANOVA results for the correlation between thermal tolerance and the strength of

348 phenotypic plasticity with environmental parameters.

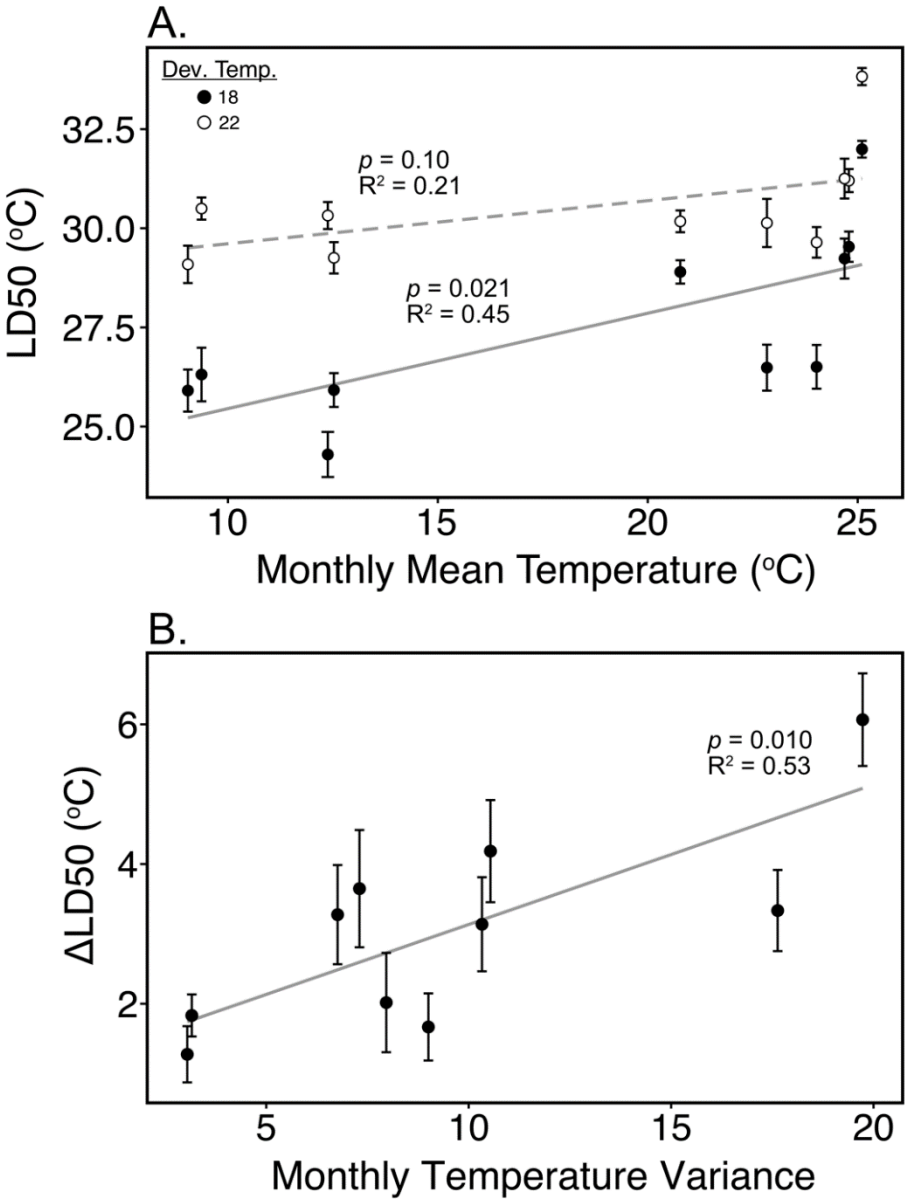
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
LD_{50}					
Dev. Temp.	1	45.923	45.923	37.1471	2.05E-05
Monthly Mean	1	25.722	25.722	20.8062	0.0003746
Monthly Maximum	1	0.693	0.693	0.5603	0.4657218
Monthly Minimum	1	19.704	19.704	15.9388	0.0011775
Residuals	15	18.544	1.236		
ΔLD_{50}					
Monthly Variance	1	10.8028	10.8028	10.3074	0.01485
Monthly Range	1	0.3555	0.3555	0.3392	0.57859
Residuals	7	7.3364	1.0481		

349



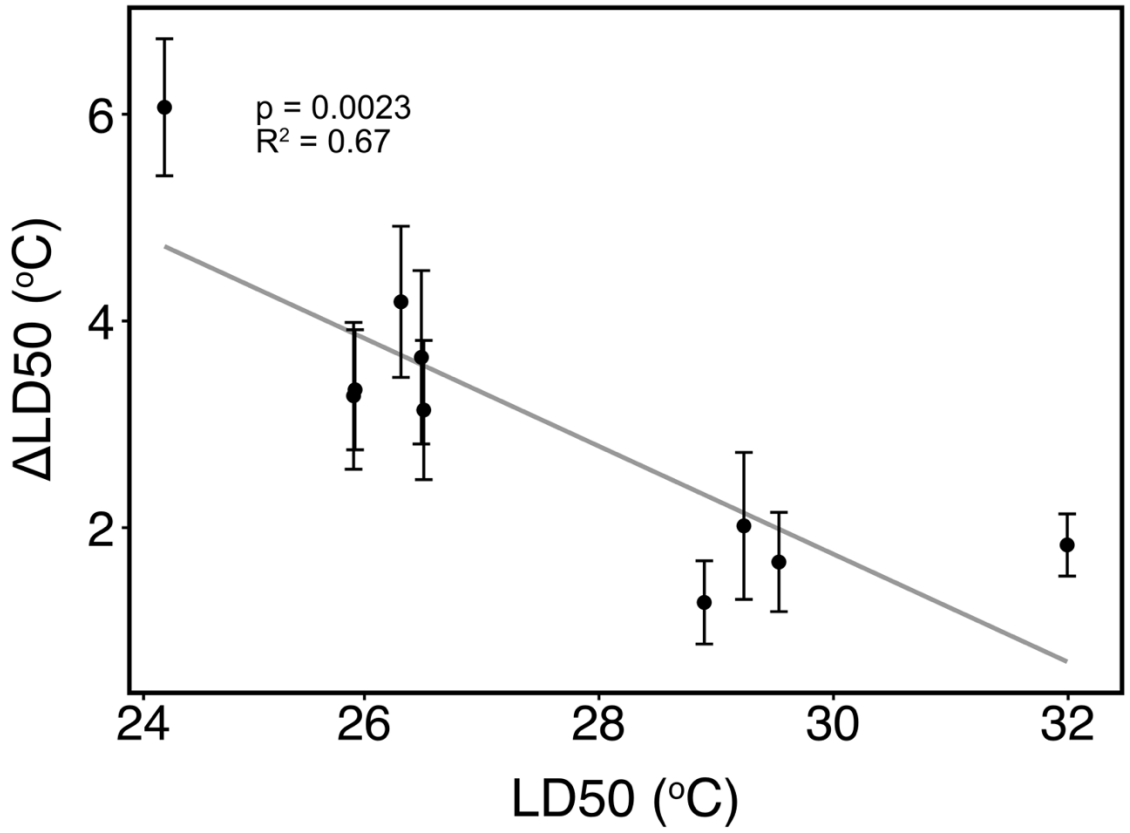
350

351 Figure 4: Latitudinal patterns in thermal adaptation. A) Thermal tolerance (LD_{50}) is plotted
352 against latitude of origin. Filled circles represent thermal tolerance values from the 18°C
353 developmental temperature while unfilled circles are values from the 22°C developmental
354 temperature. B) Developmental phenotypic plasticity (ΔLD_{50}) is plotted against latitude of
355 origin. In both panels, error bars represent standard error. Boxes highlight the latitudinal region
356 with limited divergence of thermal performance curves.



357

358 Figure 5: Correlation between thermal performance metrics and environmental parameters, as
359 predicted by the Climate Variability Hypothesis. A) Thermal tolerance (LD_{50}) is correlated with
360 mean monthly temperature. Filled circles represent thermal tolerance values from the 18°C
361 developmental temperature while empty circles are values from the 22°C developmental
362 temperature. B) Phenotypic plasticity (ΔLD_{50}) is correlated with mean monthly temperature
363 variance. In both panels, error bars represent standard error.

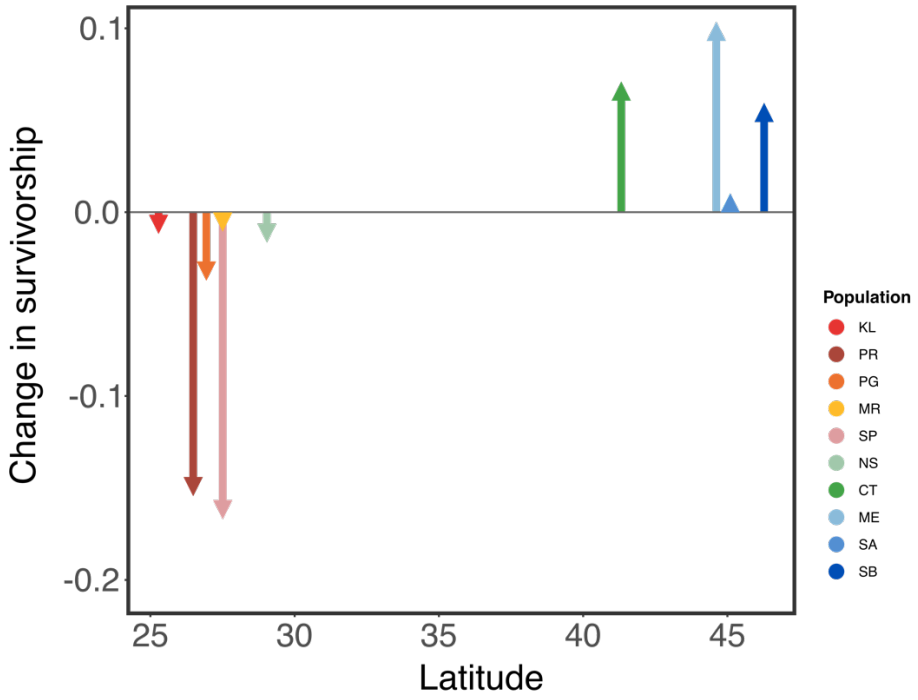


364

365 Figure 6: Linear correlation between thermal tolerance (LD_{50}) and the strength of developmental
366 phenotypic plasticity (ΔLD_{50}) for the ten populations. Error bars represent standard error.

367 Vulnerability to Warming

368 Change in survivorship at mean monthly maximum temperatures ranged from -16% to +9.8%
369 (Supp. Table 1). There was a general trend in vulnerability across latitudes (Fig. 7). Locally
370 adapted populations from low latitudes either saw almost no change (KL and MR) or a reduction
371 in survivorship (PG and PR). The locally adapted population from high latitudes (SB) saw an
372 increase in survivorship. Generally, populations without differentiation of TPCs saw a decrease
373 in survivorship at low latitudes (NS and SP) but an increase in survivorship at high latitudes (CT
374 and ME).



375

376 Figure 7: Vulnerability to predicted warming over the next century. Vulnerability is estimated as
377 the change in survivorship at the mean monthly maximum temperature if the developmental
378 temperature is assumed to be the mean monthly temperature. Positive changes represent an
379 increase in survivorship while negative changes represent a decrease in survivorship. Estimates
380 of the magnitude of warming and the temperature values used for estimating change in
381 survivorship for each population can be found in Supp. Table 1.

382 Genetic Diversity and Gene Flow

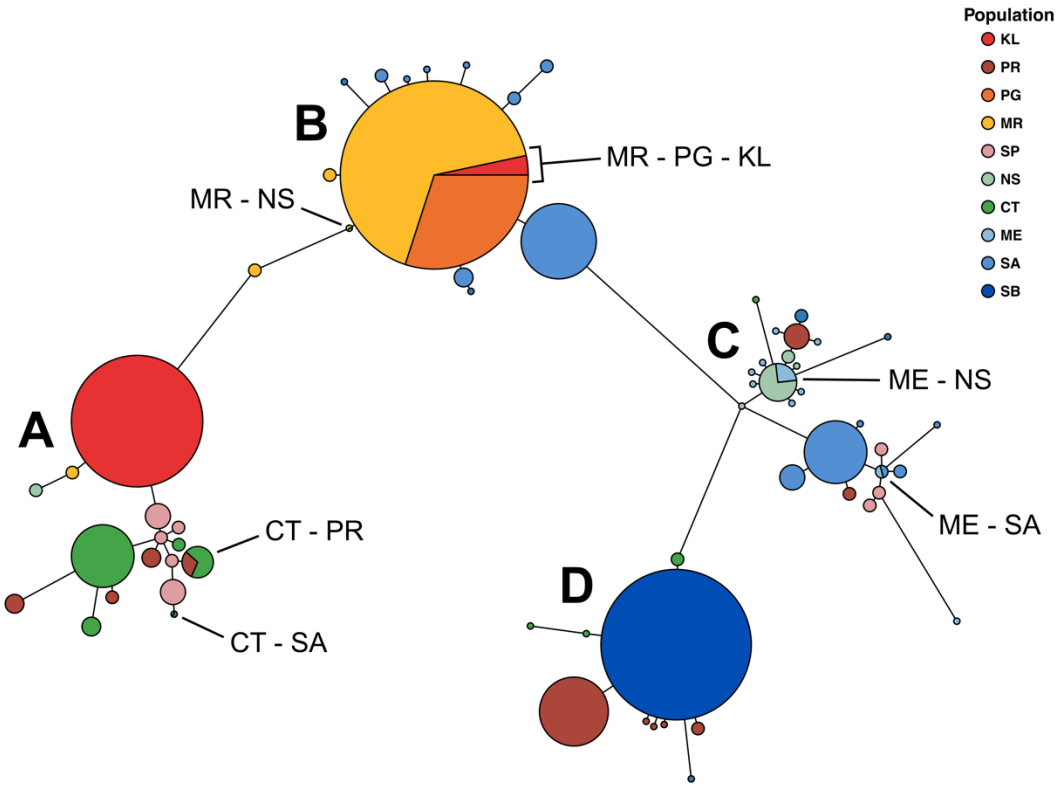
383 A total of 228 COI sequences (aligned length: 562 base pairs) were used for the genetic analyses.
384 COI sequences revealed high levels of genetic diversity (Table 5). Large variation between
385 populations was observed in haplotype diversity (H_d; 0.189 - 0.941), nucleotide diversity (π;
386 0.0029 - 0.079), and the average number of nucleotide differences between the haplotypes (1.47 -
387 40.46).

388 Table 5: Population genetic summary statistics, as calculated by DNaSP.

Population	Individuals	Haplotypes	Haplotype Diversity	Nucleotide Diversity	Average Number of Nucleotide Differences
KL	31	2	0.67527	0.00526	2.69
PR	21	11	0.85714	0.03549	18.17
PG	19	1	0.66082	0.01597	8.17
MR	34	5	0.94118	0.07903	40.46
SP	20	8	0.84211	0.0768	39.32
NS	17	6	0.83824	0.00287	1.47
CT	22	8	0.77922	0.0565	26.71
ME	20	11	0.18947	0.02072	10.61
SA	21	18	0.74286	0.03633	18.60
SB	23	3	0.74704	0.01583	8.11

389

390 In all, 73 unique haplotypes were identified, which segregated into four major clades (Fig. 8).
391 Each clade was represented in populations from across a large geographic area, and most
392 populations contained individuals from multiple clades. Additionally, we observed several
393 instances of shared haplotypes between geographically distant populations (highlighted in Fig.
394 8). As exceptions to this, the Key Largo, Punta Gorda, Manatee River, and Shediac Bay
395 populations, each of which were characterized by differentiated thermal performance curves,
396 were all largely represented by a single dominant haplotype each, which was generally not
397 recovered from other populations. The dominant single haplotype is, however, shared between
398 Punta Gorda and Manatee River. Estimates of the number of migrants from MigrateN varied
399 greatly between populations, ranging between 2.6 to 107.7 per generation. There was a negative
400 correlation between the number of migrants exchanged and the pairwise population difference in
401 LD_{50} values ($p = 0.045$; Supp. Fig. 6). However, this correlation explained only a very small
402 proportion of the variance ($R^2 = 0.07$).



403

404 Figure 8: Mitochondrial COI haplotype network for *Acartia tonsa* sequences recovered from
405 each of the ten populations. Individual circles represent unique haplotypes, with the size of each
406 proportional to the haplotype's frequency. Connecting bar lengths are proportional to the number
407 of base pair differences between haplotypes. Circles are colored based on the sequence
408 population of origin. Shared haplotypes are labelled with the sampling sites they were recovered
409 from. Four distinct clades are observed and labelled A – D.

Discussion:

Thermal tolerance and the strength of phenotypic plasticity both vary strongly among populations of *Acartia tonsa* from across the North Atlantic and Gulf of Mexico. This variation conforms to the predictions of the CVH (Janzen, 1967; Stevens, 1989), but may also be explained by Stillman's hypothesis regarding the trade-off between thermal tolerance and the strength of phenotypic plasticity (Stillman 2003). We also observed a surprising lack of differentiation of thermal performance across a large portion of the sampled range. Mitochondrial COI sequences suggest that this lack of differentiation is due to constraint by gene flow, rather than selection for a generalist performance curve. The patterns we observed in both thermal tolerance and the strength of phenotypic plasticity may result in regional differences in vulnerability to warming, with low latitude populations being the most vulnerable.

Patterns in Thermal Adaptation

Key Largo (KL), the southernmost population, had the highest thermal tolerance of the ten populations examined, followed by three populations from the Gulf of Mexico. The northernmost population, Shediac Bay (SB), had the lowest thermal tolerance but the largest strength of phenotypic plasticity. Manatee River (MR), one of the Gulf of Mexico populations with higher thermal tolerance, had the smallest strength of phenotypic plasticity. The CVH (Janzen, 1967; Stevens, 1989) predicts higher thermal tolerance in warmer environments and increased phenotypic plasticity in more variable environments. The results of our study are consistent with these predictions. We observed significant correlations between I) thermal tolerance and mean monthly temperature and II) the strength of phenotypic plasticity and mean monthly temperature variance. The observed patterns, however, may also be explained by a trade-off between thermal tolerance and phenotypic plasticity (Stillman, 2003). We observed a

433 significant negative relationship between thermal tolerance and the strength of phenotypic
434 plasticity, but no significant correlation between mean monthly temperature and mean monthly
435 temperature variance. Together, these observations might indicate that the trade-off proposed by
436 Stillman (2003) has a strong influence on shaping patterns of adaptation in this taxon, as there is
437 no covariance between environmental parameters that would drive the observed correlation
438 between LD_{50} and ΔLD_{50}. The few studies that have estimated the strength of selection on
439 phenotypic plasticity generally indicate relatively weak selection (Arnold *et al.,* 2019). It is not
440 implausible, therefore, that observed spatial patterns in phenotypic plasticity might instead be
441 driven by patterns of selection for increased thermal tolerance. However, support for this
442 hypothesis appears to be limited and species specific (Gunderson & Stillman, 2015), warranting
443 investigation in additional species.

444 The negative relationship between thermal tolerance and the strength of phenotypic plasticity
445 might reflect a role of genetic accommodation or "plasticity first" evolutionary change (Scheiner
446 *et al.,* 2017; Kelly, 2019; Levis & Pfennig, 2016; Levis *et al.,* 2017; Pfennig *et al.*, 2010;
447 Pigliucci *et al.,* 2006; Price *et al.*, 2003; West-Eberhard, 2005; 2003), wherein phenotypic
448 modification by plasticity becomes canalized or fixed by genetic mechanisms, resulting in the
449 loss of capacity for plastic change. The Manatee River - St. Petersburg (MR - SP) comparison
450 best illustrates this in our study. These sites are in close proximity to each other (a feature
451 discussed further below) but exhibit strong differences in both thermal tolerance and the strength
452 of phenotypic plasticity. St. Petersburg had a lower thermal tolerance in the 18°C developmental
453 temperature group, but larger strengths of phenotypic plasticity than Manatee River. Increasing
454 developmental temperature reduces the difference in thermal tolerance between the two
455 populations. If we assume that these two populations share an ancestral phenotype similar to the
456 contemporary St. Petersburg population, the elevated thermal tolerance but reduced plasticity in

Manatee River may reflect the fixation of changes originally induced by phenotypic plasticity in the Manatee River population. However, this is the only population pair where this is observed (the Punta Gorda (PG), Punta Rasa (PR), and Key Largo (KL) populations all had comparable or higher thermal tolerance values but larger strengths of phenotypic plasticity than the Manatee River population), suggesting that there may be several alternative evolutionary mechanisms at play across larger spatial scales.

Spatial Scales of Adaptation - Gene flow vs. Selection

Local adaptation within the range of dispersal, so called microgeographic adaptation (Richardson *et al.,* 2014), has garnered increased attention. "Microgeographic" may be a misleading term in marine systems though, as dispersal kernels can encompass hundreds of kilometers (Cowen & Sponaugle, 2009; Kinlan & Gaines, 2003; Kinlan *et al.,* 2005). The lack of differentiation we observed over a large geographic area, ranging from the Gulf of Mexico to the Bay of Fundy, is in agreement with the expectation of high levels of gene flow in pelagic copepods and other planktonic taxa. The wide distribution of genetic clades, several instances of shared haplotypes between distant populations, and the negative correlation between number of migrants and the pairwise difference in LD_{50} suggests that gene flow can be strong enough to constrain the adaptive divergence of thermal performance curves in this taxon. Direct dispersal across these large distances within the short generation times of *A. tonsa* is unlikely. Instead, dispersal may follow a stepping-stone model (Kimura & Weiss, 1964), as is common in other coastal and estuarine taxa (Hellberg, 1995; Burridge *et al.*, 2004; Williams *et al.*, 2008; Ragionieri *et al.*, 2010; Crandall *et al.*, 2012).

In stark contrast to this pattern of long-distance connectivity, we also observed significant differentiation of TPCs over spatial scales of less than ten kilometers, suggestive of

480 microgeographic adaptation. Copepods from the Manatee River (MR) and St. Petersburg (SP)
481 sites were collected from either side of a strong salinity gradient, with the salinity at the St.
482 Petersburg collection site approaching full oceanic levels (32 psu) and the Manatee River site
483 strongly influenced by riverine input (5 psu). Adaptation to salinity has been shown to
484 correspond with reproductive isolation in other populations of *Acartia tonsa* (Plough *et al.*,
485 2018). In this case, dispersal and gene flow may be strongly limited by this salinity gradient,
486 allowing for the local adaptation of the thermal performance curve in the Manatee River
487 population, while the St. Petersburg population remains under the constraining influence of gene
488 flow from other environments. This is supported by the population genetic data; the Manatee
489 River individuals are almost entirely represented by a single haplotype in Clade B, while St.
490 Petersburg individuals are represented by several different haplotypes in clades A, C, and D. No
491 haplotypes are shared between these two sites, despite their distinct geographic proximity. It
492 should also be noted that the major haplotype found at Manatee River represents the only
493 haplotype found at Punta Gorda (PG), the other low salinity site included in this study. However,
494 unlike in the Manatee River - St. Petersburg comparison, the Punta Gorda (PG) and the adjacent
495 Punta Rasa (PR) populations share a similar TPC, despite being genetically distinct. The
496 apparent local adaptation of both populations suggests that factors other than salinity may also
497 contribute to isolation and the reduction of gene flow between Punta Rasa and other sites.

498 Interestingly, differentiation of TPCs occurs predominantly within clades rather than between
499 clades, suggesting a rapid rate of thermal adaptation relative to the differentiation of the COI
500 marker region. Each clade contains several distinct thermal phenotypes. Clade A for example
501 contains populations representing three of the distinct groups of TPCs. This appears to be true for
502 both temperature and salinity conditions, as clade B is recovered from warm and fresh sites like
503 Manatee River (MR) and Punta Gorda (PG) as well as cold and high salinity sites like St.

Andrew (SA). The generally large capacity for plasticity observed in *Acartia tonsa* may promote this wide distribution of clades across environmental conditions. Phenotypic plasticity may allow migrants to survive in a wider range of environments, thus increasing gene flow and constraining local adaptation (Crispo, 2008; Thibert-Plante & Hendry, 2011).

Of course, as is the case for all single-gene markers studies, our results must be interpreted with caution. Single-gene markers are vulnerable to incomplete lineage sorting (Nichols, 2001), and may obscure important patterns in genetic structuring. Genome-scale data would provide many more markers for population genomic assessment of structure and gene flow, but for the purposes of this study COI sequences provide robust evidence for where, across the examined range, gene flow may be potentially restricting adaptive divergence of performance curves.

Vulnerability to Climate Change

Because copepods are key components in marine ecosystems and biogeochemical cycles determining their susceptibility to warming is essential for predicting the fate of the oceanic biota in light of climate change. An increasingly large body of literature has recognized the important role phenotypic plasticity may play in determining organismal responses to rapid climate change (Somero, 2010). Much of this literature has focused on acclimation or hardening, but our results show clearly that developmental phenotypic plasticity also deserves increased scrutiny as a factor affecting vulnerability (Burggren, 2018). In most of the populations examined, we observed an almost 1:1 relationship between the increase in thermal tolerance compared with the increase in developmental temperature. Strong phenotypic plasticity like this may reduce vulnerability to rapid climate change by providing a mechanism for correspondingly rapid phenotypic change. However, developmental phenotypic plasticity had a weaker effect on thermal limits than thermal tolerance ($\Delta LD_{10} < \Delta LD_{50}$; Supp. Fig. 1). The apparent constraint of upper thermal limits

527 suggests that accommodation of future warming by plasticity alone may increase vulnerability to
528 extreme temperature events like heatwaves (Meehl, 2004; Perkins *et al.,* 2012), as the difference
529 between environmental temperature and thermal limits decreases.

530 In addition to strong phenotypic plasticity, the high levels of genetic diversity, a large potential
531 for gene flow, and the apparently rapid rate of thermal adaptation (when gene flow is weak)
532 would also suggest reduced vulnerability to warming. Selection on standing genetic variation
533 may provide a rapid response to change (Pantel *et al.,* 2015; Barrett & Schluter, 2008; Torda *et*
534 *al.,* 2017). Plasticity may also play a role in increasing or maintaining migration between
535 populations, indirectly supporting evolutionary rescue (Crispo, 2008). Paired with the high levels
536 of standing genetic diversity observed both within and between populations, increasing migration
537 success may reduce vulnerability. This makes understanding oceanographic effects of climate
538 change an important prerequisite for predicting biotic responses, as changes in ocean current
539 patterns may strongly affect the potential for gene flow between populations. Reductions in gene
540 flow may promote local adaptation, thus reducing vulnerability, while an increase in gene flow
541 could strongly increase vulnerability to climate change if existing local adaptation is eroded by
542 gene swamping (Lenormand, 2002).

543 Vulnerability will also be affected by the pre-existing spatial patterns in adaptation. Using the
544 current and predicted temperatures at each site, we observed large variation in the potential
545 change in survivorship at maximum temperatures. In the southern range, warming generally had
546 little effect on survivorship at the maximum temperature in locally adapted populations, likely
547 due to accommodation by developmental phenotypic plasticity. While the response was mixed,
548 non-differentiated populations (those constrained by gene flow) may be strongly affected by
549 warming; the St. Petersburg (SP) population saw the largest decrease in survivorship. Both non-

differentiated and locally adapted populations from higher latitudes generally saw increased survivorship. Stronger predicted warming in this region may drive a larger increase in thermal tolerance due to developmental phenotypic plasticity, especially in the northernmost population Shediac Bay (SB), which had the largest strength of phenotypic plasticity. In general, local adaptation to increased temperature does not appear to increase vulnerability to warming, contrary to what has been previously suggested for warm-adapted tropical species (Somero, 2010; Tewksbury *et al.*, 2008; Nguyen *et al.*, 2011). However, this is likely highly regionally specific; the second largest decrease in survivorship was predicted for one of the locally adapted populations, Punta Rasa (PR), from the Gulf of Mexico.

Our analysis incorporates population differences in both thermal tolerance and the strength of phenotypic plasticity to provide a more robust estimate of vulnerability. Previous work also indicated that warm-adapted low latitude populations may be more vulnerable to warming, as they already experience temperatures near their thermal limits (Sasaki *et al.*, 2019). This study refines that prediction with the inclusion of more populations from a wider range of thermal environments, and the integration of population genetic data. However, our analysis cannot account for other important factors that will also likely play a large role in determining vulnerability to climate change. Factors such as changing food quality and quantity (Gregg *et al.*, 2003; Van der Waal *et al.*, 2010; Paul *et al.*, 2015; Hixon & Arts, 2016; Dutkiewicz *et al.*, 2019), changes in predation pressure (Broitman *et al.*, 2009; Rall *et al.*, 2009; De Block *et al.*, 2013; Allan *et al.*, 2015), phenological mismatches between copepods, their prey, and their predators (Edwards & Richardson, 2004; Søreide *et al.*, 2010; Brown *et al.*, 2016), changes in behavior (Kearney *et al.*, 2009; Marshall *et al.*, 2013; Nagelkerken & Munday, 2015), and changing direction or magnitude of gene flow between populations all might strongly shape population vulnerability to climate change. Our analysis also assumes that populations are not undergoing

574 range shifts or further evolutionary adaptation. Despite these limitations, our results provide a
575 representative baseline estimate of vulnerability incorporating several different adaptive
576 mechanisms.

577 The major conclusions of this study are possible only through the integration of physiological
578 experiments and molecular ecology. On their own, the thermal performance curves cannot
579 differentiate the potential explanations for the lack of divergence observed across large distances,
580 selection for a generalist performance curve or gene flow over evolutionary timescales. The
581 population genetic insights alone are not enough to infer vulnerability. We demonstrate that tight
582 integration between different fields can provide a more comprehensive understanding of the
583 factors determining vulnerability to climate change.

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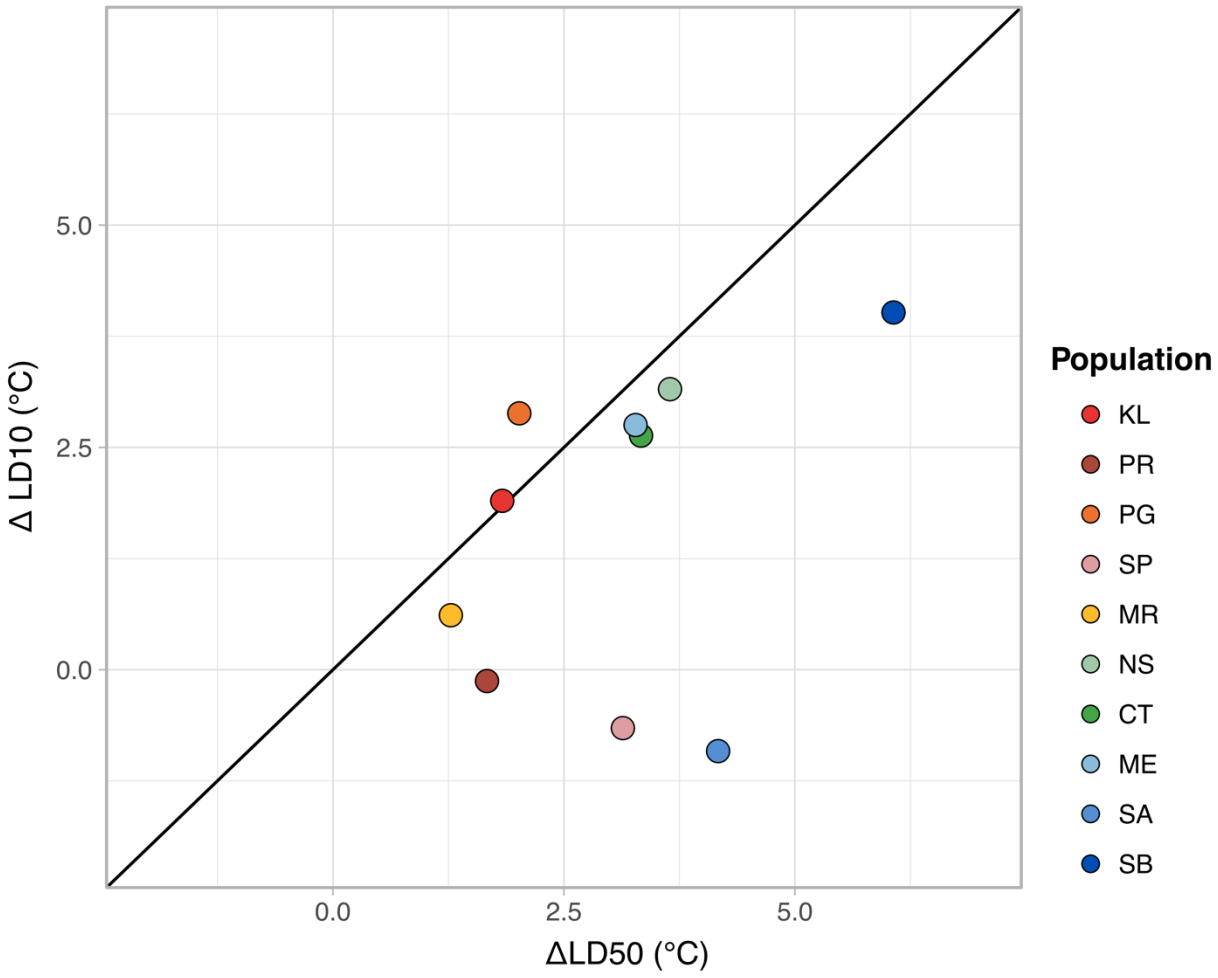
1020

Supplementary Table

Supp. Table 1: Temperature data used for predictions of vulnerability to warming. Current temperature data was acquired from the MODIS-aqua SST database. Warming was estimated from a high-resolution model of warming in the North Atlantic (Saba *et al.* 2016). Change in survivorship at maximum temperatures was estimated using TPCs based on a developmental temperature equal to the mean temperature.

Pop	Latitude	Current Mean Temp (°C)	Current Max Temp (°C)	Future Mean Temp (°C)	Future Max Temp (°C)	Estimated Temp Increase (°C)	Change in Survivorship
KL	25.283891	25.1	29.56	26.6	31.06	1.5	-0.0023
PR	26.483536	24.79	29.42	26.29	30.92	1.5	-0.15
PG	26.940398	24.69	28.8	26.19	30.3	1.5	-0.032
MR	27.505606	20.77	21.84	22.27	23.34	1.5	-0.0049
SP	27.507322	24.02	29.5	25.52	31	1.5	-0.16
NS	29.037478	22.84	26.71	24.34	28.21	1.5	-0.011
CT	41.320591	12.52	18.69	14.52	20.69	2	0.065
ME	44.615086	9.05	11.87	12.55	15.37	3.5	0.098
SA	45.100059	9.37	13.06	12.87	16.56	3.5	3.50E-04
SB	46.27291	12.38	15.76	13.88	17.26	1.5	0.054

1033 **Supplementary Figures**

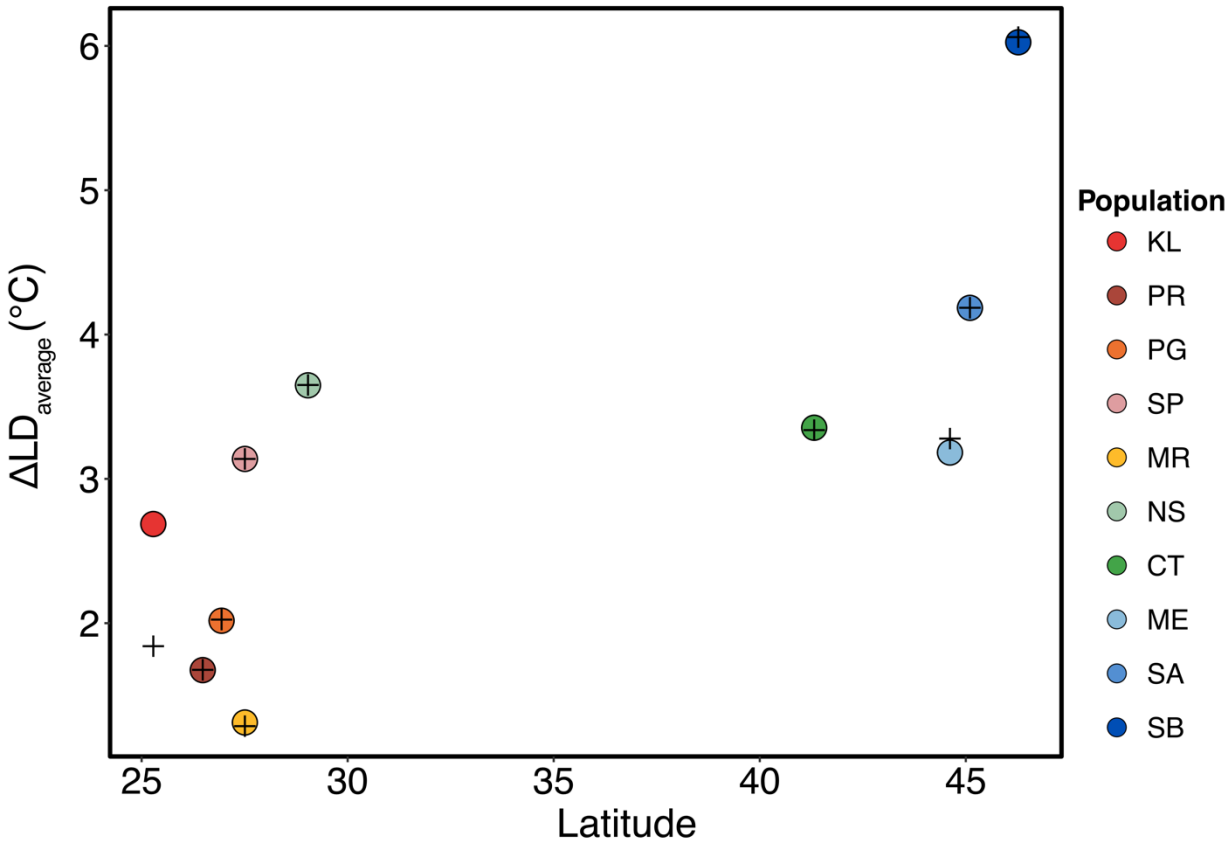


1034

1035 Supp. Fig. 1: Change in thermal limits vs. change in thermal tolerance by developmental

1036 phenotypic plasticity. The solid line represents a 1:1 relationship. Points that fall below the line

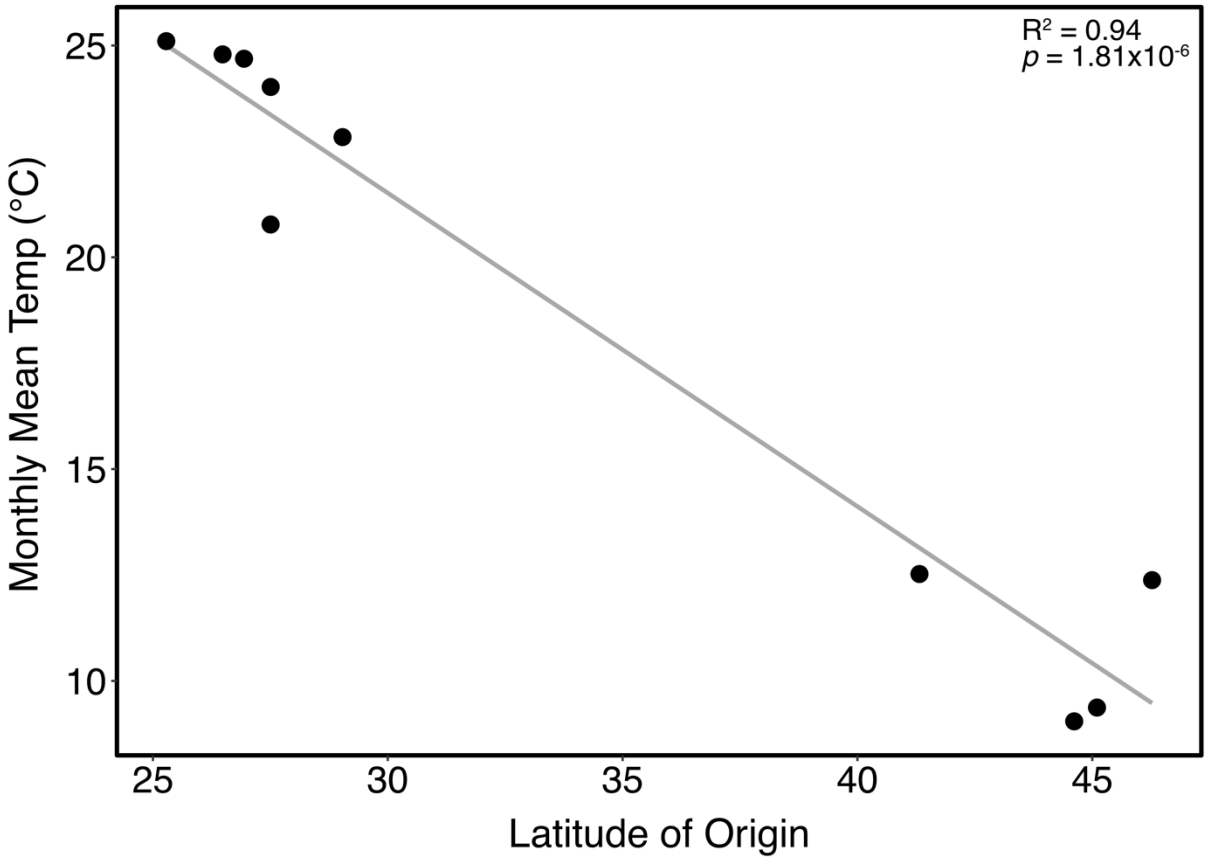
1037 represent a larger increase in thermal tolerance than thermal limits.



1038

1039 Supp. Fig. 2: Average increase in survivorship at every dosage level. No major differences are

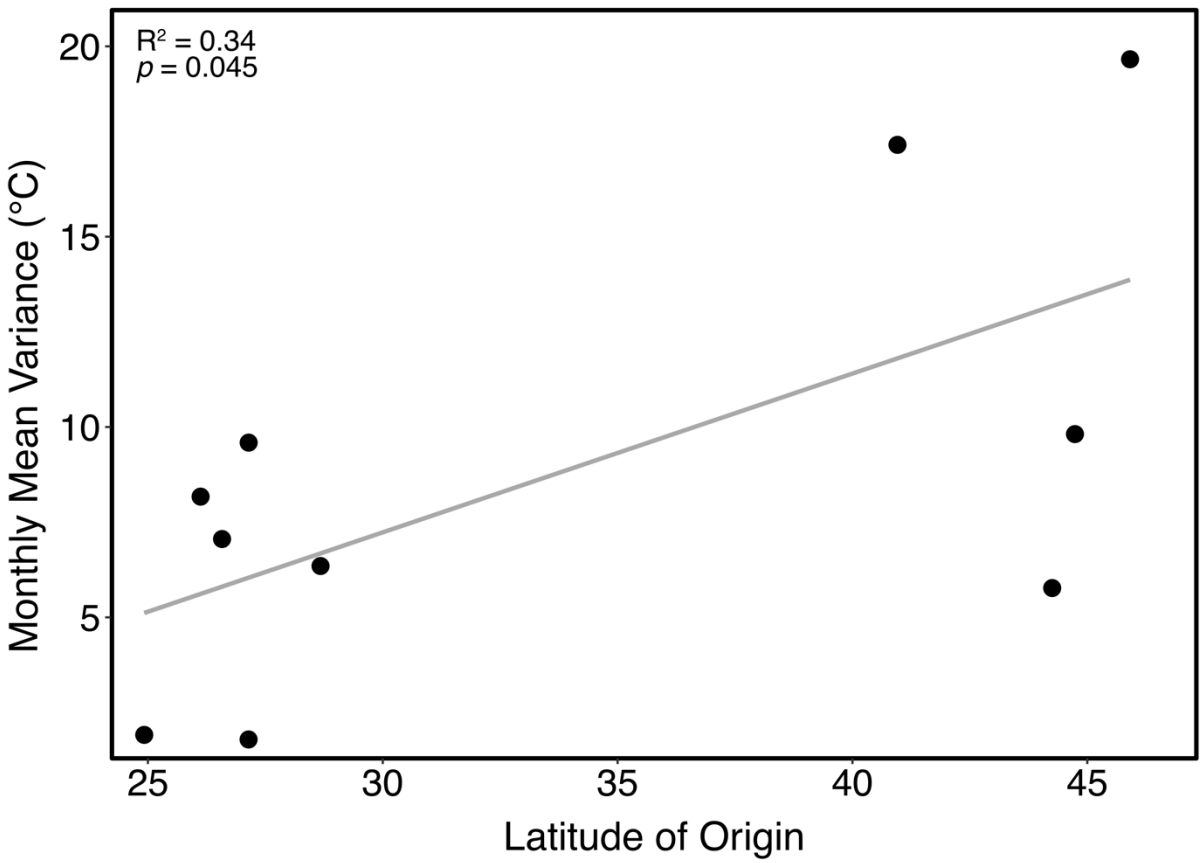
1040 observed between this metric and ΔLD_{50} values, which are represented by black crosses.



1041

1042 Supp. Fig. 3: Correlation between monthly mean temperature and latitude. A significant

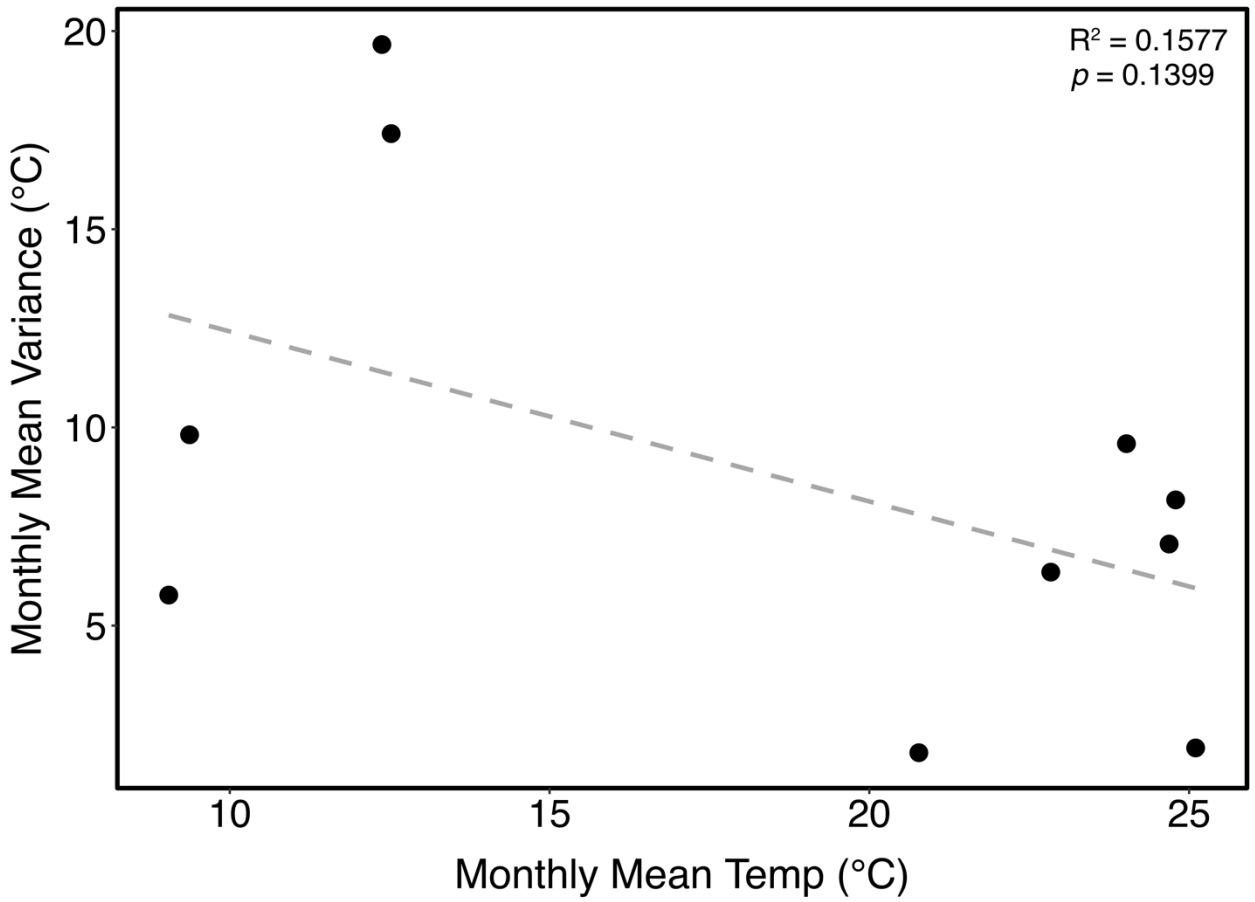
1043 correlation is observed.



1044

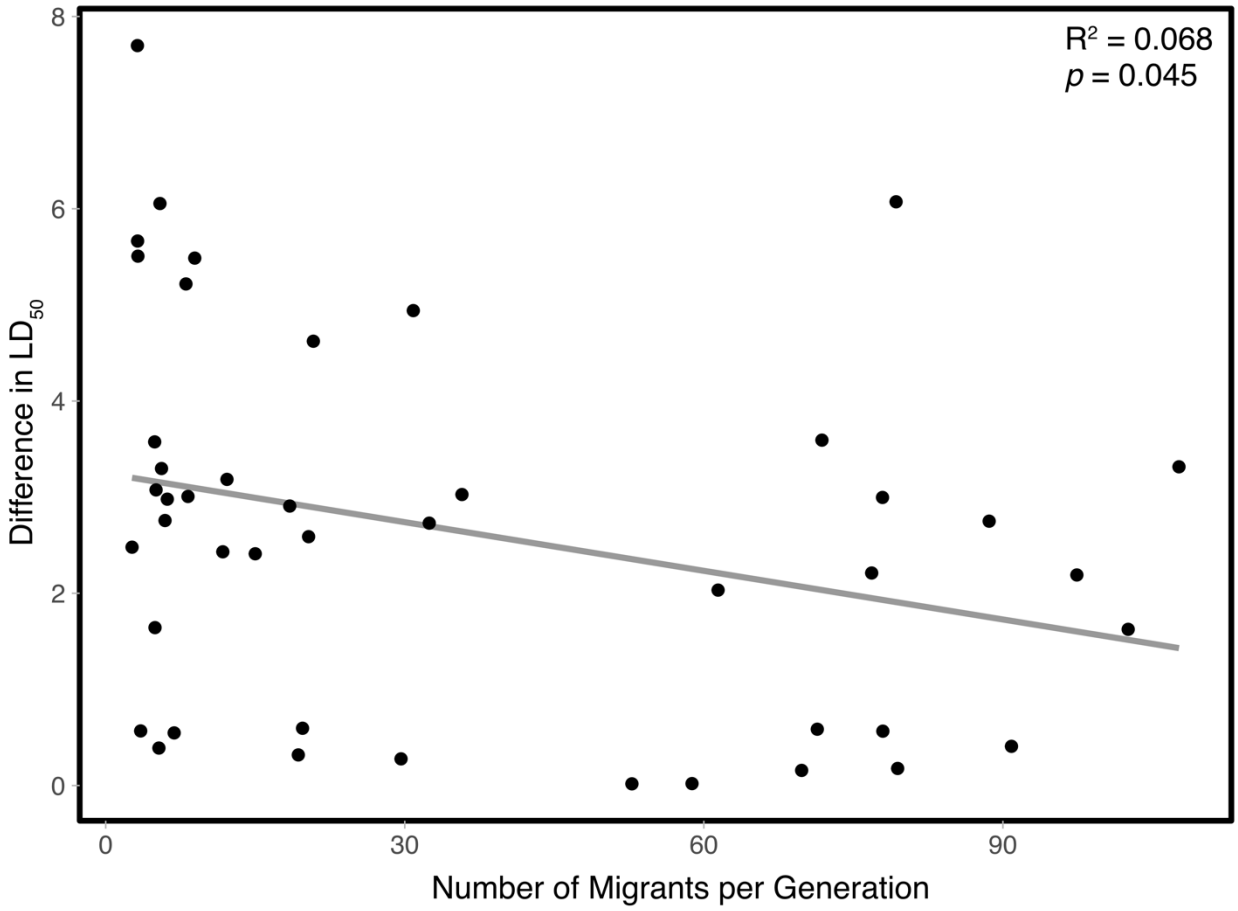
1045 Supp. Fig. 4: Correlation between monthly mean temperature variance and latitude. A significant

1046 correlation is observed.



1047

1048 Supp. Fig. 5: Correlation between the two explanatory environmental variables (mean monthly
1049 temperature and mean monthly temperature variance). No significant relationship between the
1050 variables is observed.



1051

1052 Supp. Fig. 6 – Correlation between the estimated number of migrants exchanged between two

1053 populations and the corresponding pairwise difference in thermal tolerance values between those

1054 populations.