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Positive genetic covariance and limited thermal tolerance constrain tropical insect responses to global warming

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Abstract

Tropical ectotherms are particularly vulnerable to global warming because their physiologies are assumed to be adapted to narrow temperature ranges. This study explores three mechanisms potentially constraining thermal adaptation to global warming in tropical insects: (a) Trade-offs in genotypic performance at different temperatures (the jack-of-all-trades hypothesis), (b) positive genetic covariance in performance, with some genotypes performing better than others at viable temperatures (the 'winner' and 'loser' genotypes hypothesis), or (c) limited genetic variation as the potential result of relaxed selection and the loss of genes associated with responses to extreme temperatures (the gene decay hypothesis). We estimated changes in growth and survival rates at multiple temperatures for three tropical rain forest insect herbivores (*Cephaloleia* rolled-leaf beetles, Chrysomelidae). We reared 2,746 individuals in a full sibling experimental design, at temperatures known to be experienced by this genus of beetles in nature (i.e. 10–35°C). Significant genetic covariance was positive for 16 traits, supporting the 'winner' and 'loser' genotypes hypothesis. Only two traits displayed negative cross-temperature performance correlations. We detected a substantial contribution of genetic variance in traits associated with size and mass (0%–44%), but low heritability in plastic traits such as development time (0%–6%) or survival (0%–4%). Lowland insect populations will most likely decline if current temperatures increase between 2 and 5°C. It is concerning that local adaption is already lagging behind current temperatures. The consequences of maintaining the current global warming trajectory would be devastating for tropical insects. However, if humans can limit or slow warming, many tropical ectotherms might persist in their current locations and potentially adapt to warmer temperatures.

KEYWORDS
broad-sense heritability, *Cephaloleia*, Chrysomelidae, climate change, elevational gradient, rolled-leaf beetles

1 | INTRODUCTION

Most tropical ectotherms are hypothesized to be thermal specialists due to both physiological and ecological constraints. From a physiological perspective, metabolic processes of tropical ectotherms are adapted to a narrow range of temperatures compared to temperate species (Angilletta, 2009). Such narrow thermal tolerances are assumed to be the product of local adaptation to constant local temperatures in the tropics (i.e. the mountain passes hypothesis, Janzen, 1967). It is suggested that narrow thermal limits, combined with restricted geographic distributions, are two key factors increasing the vulnerability of tropical ectotherms

to global warming (Mammola et al., 2019; Scheffers et al., 2017; Sheldon et al., 2018). These conclusions are based on physiological responses to extreme temperatures that are seldom experienced by organisms in nature (Araújo et al., 2013; García-Robledo & Baer, 2021; García-Robledo et al., 2016; Meza-Parral et al., 2020; Mitchell & Hoffmann, 2010; Sheldon et al., 2018; Sinclair et al., 2016). To determine how tropical ectotherms will respond to global warming, we need information on the performance of traits associated with growth and survival at different temperatures, the genetic variation for such traits and a better understanding of genotype responses to novel conditions.

During the Paleocene–Eocene Thermal Maximum (PETM) at 55.8 Ma, tropical insects experienced temperatures 4–7°C beyond those prevalent in current ecosystems (Gingerich, 2006; Wappler et al., 2012). If tropical insects retained plesiomorphic characters associated with PETM temperatures, one possibility is that they are already preadapted to temperatures beyond those experienced in their lifetime (Hansen et al., 2006). It is also possible that as proposed by the 'mountain passes' hypothesis, insect populations are locally adapted, and insect thermal safety margins are close to ambient temperatures (Araújo et al., 2013; Atkinson, 1994; Deutsch et al., 2008).

The failure or success of population persistence in novel conditions will depend on both the intrinsic thermal limits and the genetic variation available in the population (Futuyma & Moreno, 1988; García-Robledo & Horvitz, 2012). If tropical ectotherms are living at temperatures close to their upper thermal limits, a small increase in average temperatures may result in local extinctions (García-Robledo & Baer, 2021). In this study, we test three potential genetic mechanisms affecting thermal niche breadths and the responses of insect genotypes to projected global warming.

One mechanism that may promote thermal specialization is trade-offs in performance between temperatures (the 'Jack of all trades, master of none' hypothesis, Huey & Kingsolver, 1989; MacArthur, 1972). This hypothesis assumes that having a broad thermal range has a physiological cost and reduces maximum performance at any temperature. Therefore, genotypes with superior performance at a particular temperature will suffer a reduction in performance at alternative temperatures (Kingsolver et al., 2004; Mori & Kimura, 2008). One prediction of the 'Jack of all trades' hypothesis is that the covariance of genotype performance at different temperatures will be negative, promoting thermal specialization (Falconer & Mackay, 1996; Hoffmann, 2010).

A second potential scenario is that genotypes display similar responses to changing temperatures, but some genotypes perform consistently better in all environments (the 'winner' and 'loser' genotypes hypothesis, Palaima & Spitze, 2004). If performance of some genotypes is better than others in all environments, the genetic covariance of performance is expected to be positive. A 'winner' and 'loser' genotypes scenario will promote broader thermal niches and generalization (García-Robledo & Horvitz, 2012).

A third alternative mechanism is gene decay. If environmental stability relaxes selection on genes that would be needed in extreme conditions, they may rapidly become nonfunctional (Hoffmann, 2010; Maughan et al., 2007; Ostrowski et al., 2007). If gene decay has occurred, genetic covariance will not be detected (García-Robledo & Horvitz, 2012).

We tested these three genotypic response scenarios using a quantitative genetics framework (Falconer & Mackay, 1996; García-Robledo & Horvitz, 2012). For three species of neotropical beetles (Chrysomelidae: *Cephaloleia*), we estimated the genetic covariance, broad-sense heritability and thermal ranges of traits associated with development and survival. The resilience of insect populations to global warming will initially depend on their performance at increasing temperatures. However, if insects are not preadapted, adaptation to novel environments will depend on the genetic variation and responses of genotypes to global warming.

2 | METHODS

2.1 | Study site and species

We performed these experiments at La Selva Biological Station (hereafter La Selva) from September 2017 to November 2018. La Selva is a lowland wet forest in Costa Rica (10°26′ N, 83°59′ W) with a mean annual temperature of 25°C (Clark et al., 2003). La Selva is connected with the Barva Volcano in the Braulio Carrillo National Park. The La Selva–Barva elevational gradient is the highest elevational gradient of continuous forest in Central America, ranging from 50 m to 2,900 m elevation. The elevational gradient includes three life zones: lowland tropical rain forests, premontane and montane forests. At La Selva, 22 species of *Cephaloleia* beetles (Chrysomelidae) feed on 35 species of Zingiberales, a group of tropical plants that include bananas, gingers and spiral gingers (García-Robledo et al., 2017). This is one of the oldest plant–herbivore interaction known (Wilf et al., 2000). *Cephaloleia* beetles have been associated with Zingiberales for the last 40–60 MY (García-Robledo & Staines, 2008; Wilf et al., 2000). *Cephaloleia* beetles are also known as 'rolled-leaf beetles' because larvae and adult complete their life cycle inside the scroll formed by the young leaves of their host plants (Staines & García-Robledo, 2014). When leaves mature and unfurl, rolled-leaf beetles fly and colonize another leaf (Johnson, 2004a). The ecology and evolution of this charismatic plant–herbivore association has been studied for almost fifty years (e.g. Johnson, 2004b; McKenna & Farrell, 2006; Strong, 1977). Rolled-leaf beetles are becoming an emerging model system to determine the effects of global warming on tropical insects (Duffy et al., 2021).

We performed comparative analyses on heritability of thermal tolerance using three *Cephaloleia* species commonly found at La Selva: *C.* aff. *dilaticollis* Baly, *C.* aff. *dorsalis* Baly and *C. placida* Baly. These three beetle species were the focus of previous studies determining ecological, demographic, behavioural and genetic responses of tropical insects to novel environments (e.g. García-Robledo & Horvitz, 2011, 2012, García-Robledo et al., 2017). All

three species feed exclusively on young rolled leaves of plants in the order Zingiberales and are found from 0 to 500 m asl (García-Robledo et al., 2016). At La Selva, *Cephaloleia placida* and *C.* aff. *dilaticollis* mostly feed on *Renealmia alpinia* (Zingiberaceae) (García-Robledo et al., 2017). *Cephaloleia* aff. *dorsalis* feeds on plants in the family Costaceae. At La Selva, both larvae and adults mostly feed on *Costus malortieanus* (Costaceae) (García-Robledo et al., 2017).

2.2 | Selection of temperature treatments

We selected temperature treatments based on temperatures recorded every half an hour for four years along the La Selva-Barva elevational gradient (Clark et al., 2015; García-Robledo & Baer, 2021). We selected as our temperature treatments 10°C, 15°C, 20°C, 25°C and 30°C because they approximate to the minimum, maximum and average temperatures for the main life zones along the La Selva–Barva elevational gradient. For example, in the lowland forest where we performed this study (50–130 m elevation), the minimum, average and maximum temperatures approximate to 20°C, 25°C and 30°C. The tropical premontane forest (960 m elevation): min = 15°C, average = 20°C and max = 25 C°. The montane forests: min = 10°C, average = 15°C and max = 20°C. We also decided to include a treatment simulating maximum global warming temperatures at the end of this century in the lowlands (i.e. 35°C) (García-Robledo & Baer, 2021). These temperature treatments represent the full range of temperatures known to be experienced by *Cephaloleia* beetles along this tropical mountain. Because we did not know a priori the thermal boundaries of each lowland species included in this study, we decided to rear lowland insect cohorts at temperatures typical of the lowlands (min = 20°C, average = 25°C and max = 30°C), but also at temperatures beyond environmental temperatures experienced in this life zone. As shown by our results, this was an important decision, as one of the species (*Cephaloleia placida*) can survive at temperatures colder than those experienced in the lowlands.

2.3 | Larval development and survival at different temperatures

We collected adult males and females of each beetle species, placing mating pairs in individual containers. In total, we matched 33 *C.* aff. *dilaticollis* pairs, 35 *C.* aff. *dorsalis* pairs and 15 *C. placida* pairs. Females lay two to six eggs per week (García-Robledo & Horvitz, 2011). We fed beetles ad libitum with young leaf tissue from each species' most commonly used host species and provided 10 × 10 cm squares of mature leaf tissue for oviposition. Every 48 hr, eggs were removed from the leaf tissue. Because there is evidence that multiple individuals sharing resources affect each other's performance (Blanckenhorn, 1998), we placed each egg individually in containers lined with damp filter paper and assigned identification codes that recorded their parentage. Eggs from each pair were then distributed among environmental chambers built according to the specifications given in García-Robledo et al., 2020.

Because we did not know a priori the thermal ranges of the species selected for this study, eggs were assigned to six temperatures: 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. These temperatures range from the minimum temperature experienced in montane forests by high-elevation *Cephaloleia* species, to predicted maximum temperature in the year 2100 (Flato et al., 2013; García-Robledo & Baer, 2021). After we found that eggs of these lowland species failed to hatch at 10°C and 35°C, and all larvae in 30°C died, we restricted the treatments to 15°C, 20°C and 25°C. Due to high mortality of *C.* aff. *dilaticollis* and *C.* aff. *dorsalis* larvae at 15°C, we later limited quantitative genetic experiments to 20°C and 25°C temperature treatments for these two species.

All individuals experienced in the laboratory a 12-hr/12-hr light–dark regime and 100% relative humidity and were given fresh leaf tissue every 48 hr. We systematically rotated individuals within each environmental chamber to control for microclimatic differences within the chambers. We recorded larval growth by measuring newly hatched and sixteen-day-old larvae. We photographed larvae with a digital camera (Diagnostic Instruments Inc. Model 3.2.0, Sterling Heights, MI, USA) attached to a stereoscope (Leica MZ 12s), then measured larvae to the nearest 10^{-2} mm using the program SPOT V.3.5.8 (Diagnostic Instruments, Inc.). We log-transformed the data and tested for differences in length between temperatures using one-way ANOVAs in R (R Core Team, 2016).

We measured pupal lengths and masses on the day of pupation and adult lengths and masses on the day of adult emergence. Pupae and adults were weighed on a Scientech SA 40 analytic balance with a precision of 10^{-4} g. For statistical analyses, we log-transformed all length and mass data. We also calculated larval and pupal development times for individuals that successfully transitioned to the next life stage. To test for effects of temperature on pupal length, pupal mass, adult length and adult mass, we performed one-way ANOVAs when there were three treatments and Welch's *t* tests when there were two treatments.

We also estimated larval survival by checking each larva every 48 hr, recording their dates of death or pupation. Temperature effects on larval survival were tested using Cox proportional hazard survival analyses and log-rank tests (package 'survival', Therneau, 2016). We right-censored pupated larvae, as they did not die during the life stage under analysis.

2.4 | Quantitative genetic analyses

We tracked the parentage of every individual and thus can assign individuals to maternal genetic families, which represent a mix of half- and full siblings. We cannot guarantee that all offspring in the same family are full siblings because females may have stored sperm from precollection matings. We used the mix of half- and full siblings to estimate heritability values and report the raw variance components, which include an unknown combination of additive, nonadditive

and maternal effects. Because we have no paternity estimates of the randomly paired field-collected beetles, we expect the brood to be primarily full rather than half-sibs. Since full sibs have more in common that just genetic effects, our experiments most likely overestimate heritability. Therefore, for a more conservative interpretation, we suggest that all raw variance estimates must be multiplied by two, to calculate broad-sense heritability (H^2) rather than by four, which assumes that genetic variance estimates approximate to narrow-sense heritability (h^2) (Blanckenhorn, 1998, see estimates of heritability in Appendix S1). We also grouped individuals by genetic family and tested for family effects on development and survival at different temperatures. When a family had trait data for fewer than five individuals in a treatment, we omitted that family from the trait analysis (see sample sizes in Table 1).

2.4.1 | Genotype × environment interactions in development and survival traits

To test whether *Cephaloleia* species display statistically significant genetic variation in developmental traits, we compared family trait means at different temperatures for newly hatched larval length, two-week-old larval length, pupal length, adult length, pupal mass, adult mass, larval development time and pupal development time. Length and mass traits were log-transformed.

For all developmental traits, we modelled differences among families, temperatures and family–temperature interactions using mixed effects models (package 'lme4', Bates et al., 2015). Because the values of the explanatory variable (in this case, family) were not randomly sampled but deliberately chosen, and we were interested in estimating the effects of those particular families on each trait, all models included fixed interactions (Crawley, 2013).

As we recognized that the families used in this experiment are randomly sampled from their populations but were also interested in how these particular families responded to temperature, we followed the following methods (Crawley, 2013): For each trait and species, we compared mixed effects models with family as a random effect and fixed effects of temperature, family and a temperature–family interaction. Due to these different fixed effect structures, we employed maximum likelihood and compared models using log-likelihood ratio tests (Crawley, 2013). For the traits which displayed significant family–temperature interactions, we also plotted family mean reaction norms.

For survival, we modelled differences among families, temperatures and family–temperature interactions using mixed Cox proportional hazards analyses. In Cox proportional hazards analysis, frailty (a random effect model for time-to-event data) represents the possibility that some groups of individuals (in this case, families) are shorter or longer lived for unknown reasons. If the frailty ×temperature interaction is significant, it means that some of the randomly sampled families in the experiment had different survival curve shapes at different temperatures, rather than simply different end points. We have included a fixed temperature effect, a random family effect and an interaction between the two. We tested the significance of the different effects by comparing models using log-likelihood ratio tests (Therneau, 2017).

2.4.2 | Contribution of genetic variation to performance at different temperatures

We estimated the amount of variation associated with differences among genetic families using linear mixed models. Genetic variation is estimated using untransformed data, including family as a random factor (Lynch & Walsh, 1998). As each family is an unknown mix of half and full siblings, heritability is a value between broad (H^2) and narrow (h^2) sense heritability estimates (García-Robledo & Horvitz, 2012; Lynch & Walsh, 1998). In this study, we report the amount of genetic variance detected for each trait, and the relative performance of such trait at a given temperature (Appendix S1).

2.4.3 | Genetic covariance of developmental and survival traits at different temperatures

We tested whether there are trade-offs between performance at different temperatures using Pearson correlations (Lynch & Walsh, 1998). For each family, we calculated the mean trait values of families at different temperatures for newly hatched larval length, two-week-old larval length, pupal length, adult length, pupal mass, adult mass, larval development time, pupal development time and larval survival (see Figure 5 for sample sizes). We then tested whether these family trait means were correlated across different temperatures using Person product–moment correlations.

3 | RESULTS

3.1 | Larval development and survival at different temperatures

3.1.1 | Larval, pupal and adult lengths

All three *Cephaloleia* species displayed significant differences in length at different temperatures (Figure 1; Table 1). Small but statistically significant differences were already apparent in newly hatched larvae. This suggests that newly hatched larval length is a very important trait to define the future of pupae and adults. These differences became more pronounced in the two-week-old larvae before decreasing in pupae and adults, although they remained statistically significant. The pupal and adult temperature responses varied more among species than the larval patterns.

For *C.* aff. *dilaticollis*, newly hatched larvae were largest at 25°C and smallest at 15°C and 30°C, with 20°C larvae forming a distinct

TABLE 1 Statistical analyses (ANOVAs and *t* tests) for the effect of temperature on developmental traits. Genetic families with less than five individuals were not included in analyses

		Sample size								
Species	**Traits**	N_{10}	N_{15}	N_{20}	N_{25}	N_{30}	N_{35}	***df***	**Statistic**	***p***
C. aff. *dilaticollis*	*Length (mm)*									
	Day 1 larvae	0	211	450	724	51	0	3, 1,432	$F = 75.37$	$\mathbf{<2 \times 10^{-16}}$
	Day 16 larvae	0	89	250	383	0	0	2, 719	$F = 414.4$	$\mathbf{<2 \times 10^{-16}}$
	Pupae	0	13	135	169	0	0	2, 314	$F = 12.38$	$\mathbf{6.68 \times 10^{-6}}$
	Adults	0	5	88	70	0	0	143.3	$t = 3.759$	**.000248**
	Mass (g)									
	Pupae	0	13	135	169	0	0	2, 314	$F = 20.32$	$\mathbf{5.03 \times 10^{-9}}$
	Adults	0	5	88	70	0	0	152.1	$t = 2.47$	**.0147**
	Development time (days)									
	Larvae	0	13	132	164	0	0	2	$F = 283.7$	$\mathbf{<2 \times 10^{-16}}$
	Pupae	0	5	89	69	0	0	269.3	$t = 10.38$	$\mathbf{<2.2 \times 10^{-16}}$
C. aff. *dorsalis*	*Length (mm)*									
	Day 1 larvae	0	117	281	298	65	0	3, 757	$F = 30.41$	$\mathbf{<2 \times 10^{-16}}$
	Day 16 larvae	0	55	228	237	7	0	3, 523	$F = 272.5$	$\mathbf{<2 \times 10^{-16}}$
	Pupae	0	18	219	218	0	0	2, 452	$F = 12.73$	$\mathbf{4.19 \times 10^{-6}}$
	Adults	0	4	146	116	0	0	251.7	$t = 0.127$	.899
	Mass (g)									
	Pupae	0	18	219	218	0	0	2, 452	$F = 4.749$	**.0091**
	Adults	0	4	146	116	0	0	242.6	$t = -0.096$	.185
	Development time (days)									
	Larvae	0	18	218	216	0	0	2, 449	$F = 536.5$	$\mathbf{<2 \times 10^{-16}}$
	Pupae	0	5	148	117	0	0	401.9	$t = 8.421$	$\mathbf{6.7 \times 10^{-16}}$
C. placida	*Length (mm)*									
	Day 1 larvae	0	178	95	107	82	0	3, 457	$F = 23.98$	$\mathbf{1.99 \times 10^{-14}}$
	Day 16 larvae	0	133	85	93	18	0	3, 325	$F = 280.2$	$\mathbf{<2 \times 10^{-16}}$
	Pupae	0	67	82	89	0	0	2, 235	$F = 8.407$	**.00298**
	Adults	0	46	76	75	0	0	2, 194	$F = 7.336$	**.00848**
	Mass (g)									
	Pupae	0	67	82	89	0	0	2, 235	$F = 14.14$	$\mathbf{1.59 \times 10^{-6}}$
	Adults	0	46	76	75	0	0	2, 194	$F = 13.52$	$\mathbf{3.18 \times 10^{-6}}$
	Development time (days)									
	Larvae	0	67	81	89	0	0	2, 234	$F = 891$	$\mathbf{<2 \times 10^{-16}}$
	Pupae	0	48	75	77	0	0	2, 197	$F = 2{,}299$	$\mathbf{<2 \times 10^{-16}}$

intermediate group (Figure 1a; Table 1). Two-week-old larvae followed a similar pattern, although all 30°C larvae died (Figure 1b). However, pupae were longer at 20°C than at the other two temperatures (Figure 1c). Adults were longer at 20°C than at 25°C (Figure 1d).

For *C.* aff. *dorsalis*, newly hatched larvae were larger at 20°C and 25°C than at the extreme temperatures (Figure 1e; Table 1). The longest two-week-old larvae were those at 25°C, whereas the shortest were those at 15°C (Figure 1f). Two-week-old larvae at 20°C and 30°C formed a third intermediate group. At 30°C, no individuals reached pupation, but the other temperatures produced pupae of different lengths, with the shortest pupae at 15°C and the longest at 25°C (Figure 1g). *Cephaloleia* aff. *dorsalis* adults did not have significantly different lengths at 20°C and 25°C (Figure 1h).

Cephaloleia placida larvae showed the same patterns as the *C.* aff. *dorsalis* larvae, with the longest newly hatched larvae at 20°C and 25°C and the longest two-week-old larvae at 25°C (Figure 1i and j; Table 1). However, *C. placida* pupae were longest at 20°C and shortest at 25°C, with the larvae at 15°C overlapping with both groups (Figure 1k). The adults showed the same pattern (Figure 1l).

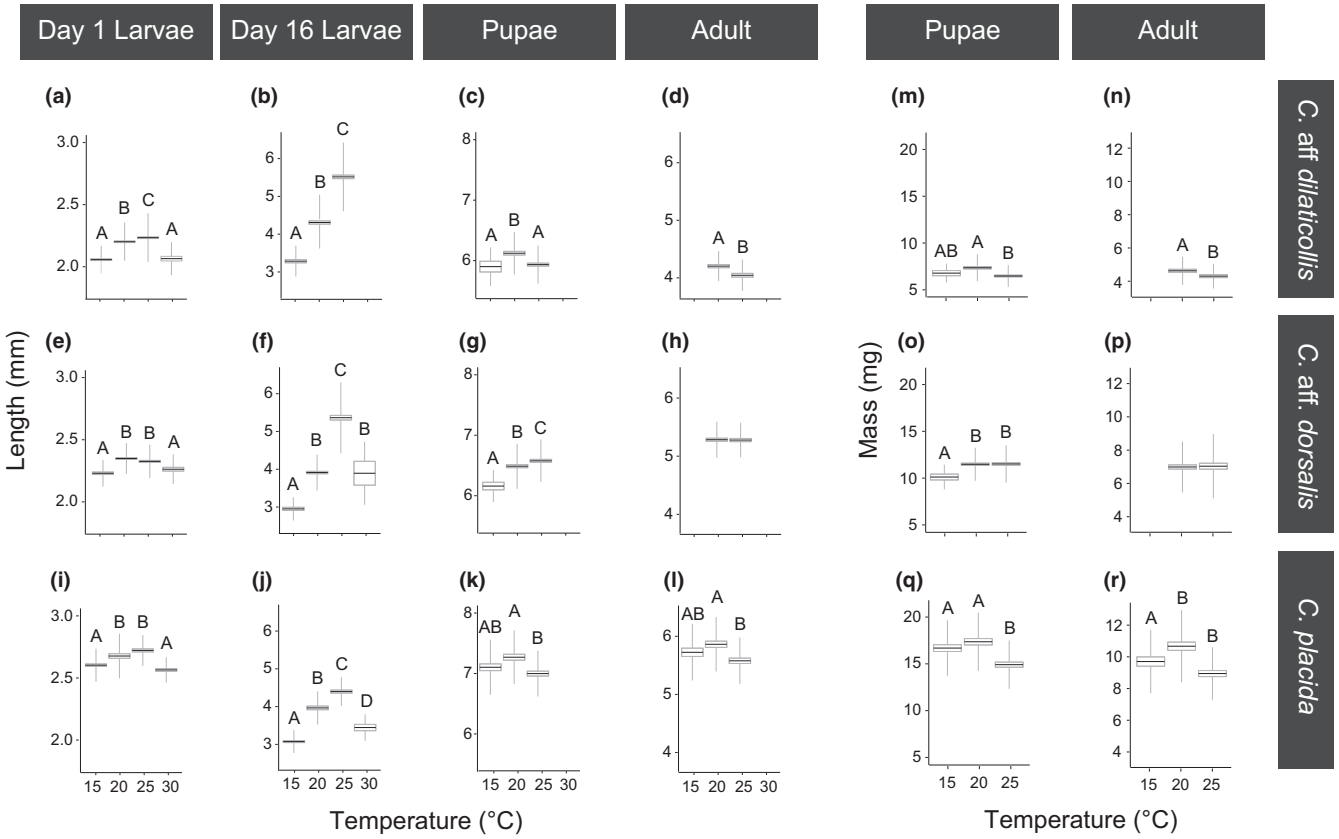

FIGURE 1 Length and mass of larvae, pupae and adults *Cephaloleia* beetles raised at multiple temperatures. (mean ± *SE* ± *SD*). Lengths: (a–d) *Cephaloleia* aff. *dilaticollis*, (e–h) *C*. aff. *dorsalis*, (i–l) *C. placida*. Masses: (m and n) *Cephaloleia* aff. *dilaticollis*, (o and p) *C*. aff. *dorsalis*, (q and r) *C. placida*. Letters indicate statistical differences between temperatures ($p < .05$, Tukey's honest significant differences). See Table 1 for sample sizes and test statistics

3.1.2 | Pupal and adult masses

The species' pupal and adult masses responded differently to temperature. For both pupae and adults of *C*. aff. *dilaticollis*, individuals reared at 20°C were heavier than individuals at 25°C (Figure 1m and n; Table 1). However, *C*. aff. *dorsalis* pupae were heavier at 20°C and 25°C and lighter at 15°C, whereas adult mass was not significantly affected by temperature (Figure 1o and p; Table 1). *Cephaloleia placida* pupae were heavier at 15°C and 20°C than at 25°C, whereas *C. placida* adults were significantly heavier at 20°C than at either 15°C or 25°C (Figure 1q and r; Table 1).

3.1.3 | Development time

All three species displayed negative relationships between temperature and development time (Figure 2; Table 1). On average, *C*. aff. *dilaticollis* larvae at 25°C developed in 42% of the time as at 15°C, whereas pupae at 25°C had a mean development time 58% of those at 20°C (Figure 2a and b). *Cephaloleia* aff. *dorsalis* larval development time at 25°C was 38% of the 15°C development time. *Cephaloleia* aff. *dorsalis* pupae at 25°C pupated in 51% of the time that those at 20°C did (Figure 2c and d). *Cephaloleia placida* had the greatest change in larval development time and the smallest change in pupal development time. *Cephaloleia placida* larvae at 25°C developed in only 32% of the time at 15°C, whereas pupae at 25°C developed in 67% of the time as pupae at 20°C (Figure 2e and f).

3.1.4 | Larval survival

In general, larval survival peaked at an intermediate temperature (20°C or 25°C) and no larvae survived at 30°C (Figure 2; Table 2). *Cephaloleia* aff. *dilaticollis* survived best at 20°C, with lower survival at 15°C and 25°C (Figure 2g). *Cephaloleia* aff. *dorsalis* survival differed at each temperature, with survival highest at 20°C and lowest at 15°C (Figure 2h). *Cephaloleia placida* larvae survived equally well at 20°C and 25°C but had lower survival at 15°C (Figure 2i).

3.2 | Quantitative genetic analyses

3.2.1 | Genotype × environment interactions in development and survival traits

We found that genetic families differed significantly in the larval traits we measured, but were less likely to differ in pupal and adult traits compared to unrelated individuals (Table 3). Our models of

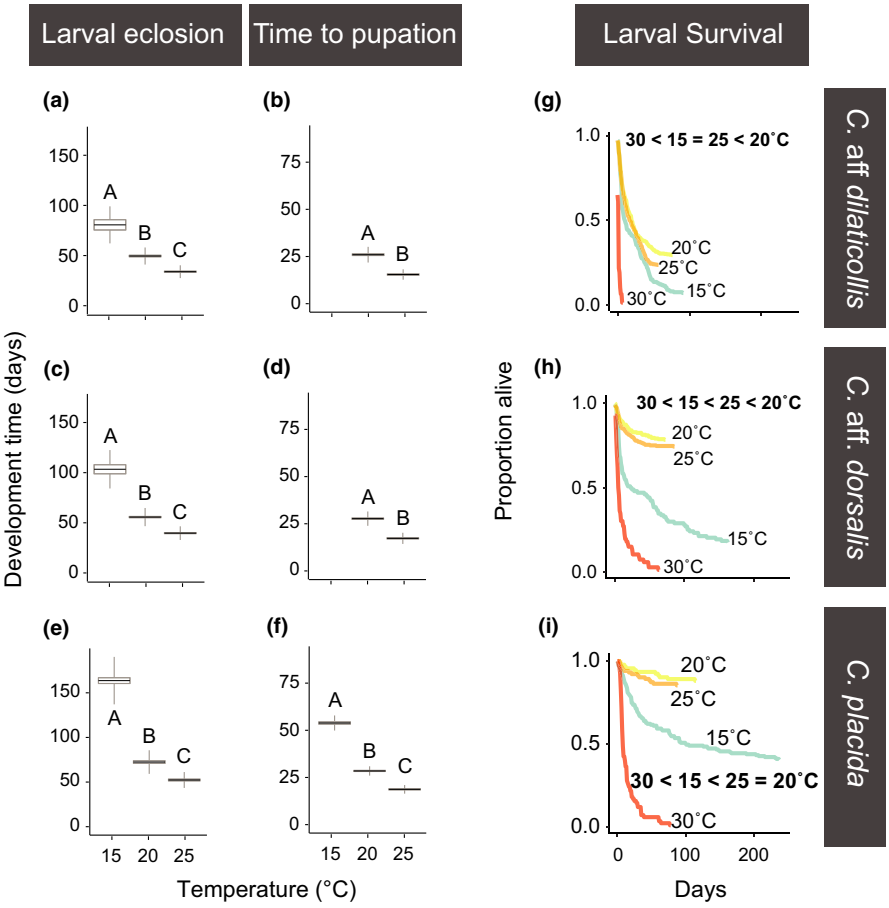

FIGURE 2 Development times (mean ± *SE* ± *SD*) and larval survival of *Cephaloleia* beetles at multiple temperatures. Development times: (a) *Cephaloleia* aff. *dilaticollis* larvae and (b) pupae, (c) *C.* aff. *dorsalis* larvae and (d) pupae, (e) *C. placida* larvae and (f) pupae. Letters indicate differences between temperatures ($p < .05$, Tukey's honest significant differences). See Table 1 for sample sizes and ANOVA statistics. Larval survival: (g) *Cephaloleia* aff. *dilaticollis*, (h) *C.* aff. *dorsalis*, (i) *C. placida*. Differences between temperatures analysed using a Kaplan–Meier log-rank significance test ($p < .05$). See Table 2 for sample sizes and test statistics

larval lengths and survival included trait data for 15°C, 20°C and 25°C (except for *C.* aff. *dorsalis* two-week-old larval length). Pupal and adult models for *C.* aff. *dilaticollis* and *C.* aff. *dorsalis* only compared 20°C and 25°C. We found three significant family ×temperature interactions, all in larval traits.

Cephaloleia aff. *dilaticollis* families differed in mean survival (Table 2). We also detected differences in newly hatched larval length, pupal length and pupal and adult masses of individuals reared at different temperatures (Table 3). There were no significant family × temperature interactions.

Cephaloleia aff. *dorsalis* families differed in mean survival (Table 2), as well as newly hatched larval length, pupal mass and larval development time (Table 3). *Cephaloleia* aff. *dorsalis* two-week-old larval length also displayed a significant family ×temperature interaction. The reaction norm for this trait showed that all families had larger larvae at 25°C than at 20°C, but their relative sizes differed between the two temperatures (Figure 3).

Cephaloleia placida families differed in newly hatched larval length and larval development time (Table 3). *Cephaloleia placida* two-week-old larval length and larval development time also displayed significant family ×temperature interactions. Reaction norms comparing families' mean two-week-old larval length showed that family size differences were consistent between 15°C and 20°C (Figure 4a), but relative lengths differed when these temperatures were compared to 25°C (Figure 4b and c). One family even produced shorter larvae at 25°C than at 20°C (Figure 4c). Relative larval development time differed between families across all treatments (Figure 4d–f).

3.2.2 | Contribution of genetic variation to performance at different temperatures

Broad-sense heritability estimates differed substantially across species, traits and temperatures (see Appendix S1 for all H^2 estimates). In general, we detected high genetic variation in length and mass when families were reared at temperatures associated with higher survival and performance (Figure S1). In some temperature treatments, variance among families can explain as much as 44% of the variation in size and mass (Figure S1). Genetic variance explains at most 8% of the variation in development traits (Figure S2a–f). Genetic variance represents a small proportion (at most 4.5%) of the total variance associated with larval survival (Figure S2g–i). Genetic variation was low, or not detected at extreme temperatures where survival and performance were low (Appendix S1).

3.2.3 | Genetic covariance of development and survival at different temperatures

We calculated 51 pairwise temperature correlations out of a potential of 81, as lower larval survival at 15°C made it challenging

TABLE 2 Comparisons of Cox proportional hazard mixed models for the larval survival of the three *Cephaloleia* species

Species	Log-likelihood	LL *df*	χ^2	χ^2 *df*	*p*
C. aff. *dilaticollis* (*N* = 33)					
Family	−7,413.0	9.81	262.4	9.81	**9.5×10^{-51}**
Temperature	−7,295.7	3	27.9	3	**3.84×10^{-6}**
Family + Temperature	−7,281.8	9.93	2.26	9.93	.99
Family × Temperature	−7,280.6	9.57			
C. aff. *dorsalis* (*N* = 35)					
Family	−1,682.2	13.33	156.9	13.33	**1.27×10^{-26}**
Temperature	−1,612.3	3	17.0	3	**6.96×10^{-4}**
Family + Temperature	−1,603.7	5.93	3.95	5.93	.67
Family × Temperature	−1,601.8	5.49			
C. placida (*N* = 15)					
Family	−1,144.4	9.1	184.9	9.1	**5.60×10^{-35}**
Temperature	−1,053.8	3	2.39	3	.49
Family + Temperature	−1,052.0	0.987	−0.161	0.987	1
Family × Temperature	−1,052.1	0.00816			

Note: N indicates the number of families. The statistics for a log-likelihood ratio test comparing each simpler model to the next more complex model are reported. Both single-factor models are compared to the additive model. In the models, temperature is included as a fixed effect and Family as a random effect. A significant *p*-value means that the simpler model is significantly worse than the more complex one.

p values in bold are significant ($p < .05$).

to collect trait data for later developmental stages. We found that eighteen of those 51 correlations were significant (Figure 5). Of these, sixteen were positive correlations and two were negative. Most of the significant correlations were for larval traits.

Cephaloleia aff. *dilaticollis* families had eight significant positive correlations and seven nonsignificant correlations (Figure 5a). The positive correlations occurred in six traits: newly hatched larval length, two-week-old larval length, pupal length, pupal mass, adult mass and larval survival. Newly hatched larval length was significantly correlated across all three temperature comparisons (15°C vs. 20°C, 15°C vs. 25°C and 20°C vs. 25°C). However, only one correlation was significant for two-week-old larval length (15°C and 20°C) and larval survival (20°C and 25°C). Pupal and adult trait correlations could only be calculated between 20°C and 25°C and were significantly positive for pupal length, pupal mass and adult mass.

Cephaloleia aff. *dorsalis* families had the fewest calculable correlations due to rapid larval mortality at 15°C. *Cephaloleia* aff. *dorsalis* families had three significant positive correlations and ten nonsignificant correlations (Figure 5b). The significant positive correlations occurred in 20°C versus 25°C comparisons for newly hatched larval length, adult length and larval developmental time.

Cephaloleia placida families had two significant negative correlations, five significant positive correlations and sixteen nonsignificant correlations (Figure 5c). The significant correlations occurred in three traits: newly hatched larval length, two-week-old larval length and larval development time. Newly hatched larval lengths showed significant positive correlations in all three temperature comparisons. However, two-week-old larval lengths were positively correlated between 15°C and 20°C but negatively correlated between 15°C and 25°C and 20°C and 25°C. Family means for larval development time were positively correlated between 15°C and 20°C, but were not significantly correlated between 15°C and 25°C or 20°C and 25°C.

4 | DISCUSSION

Supporting our general hypothesis that lowland insects are thermal specialists, we found that development and survival are strongly limited by temperature. Lowland *Cephaloleia* species cannot reach adulthood at temperatures beyond 25°C. Although La Selva frequently experiences temperatures above 30°C, no larvae of any species survived to pupate at 30°C. This suggests that lowland tropical insects are already experiencing temperatures close to their thermal safety margins and are not preadapted to future temperatures as suggested by other studies (Colwell et al., 2008).

Upper and lower thermal limits of *Cephaloleia* beetles are narrow (<5–10°C). Comparisons between our results and those reported in other studies is challenging because previous analyses are based on physiological responses such as heat coma or lethal temperatures (Lancaster, 2016). In contrast, our estimates are based on performance and survival of individuals exposed to different temperatures. Physiological responses to extreme temperatures invariably overestimate thermal ranges (García-Robledo & Baer, 2021). For example, global analyses estimate that thermal breadths of tropical

TABLE 3 Mixed effects models for family × environment interactions for *Cephaloleia* developmental traits at different temperatures

		C. aff. *dilaticollis*					*C.* aff. *dorsalis*					*C. placida*				
Trait	**Factor**	**LL**	***df***	χ^2	χ^2 ***df***	***p***	**LL**	***df***	χ^2	χ^2 ***df***	***p***	**LL**	***df***	χ^2	χ^2 ***df***	***p***
Length (mm)																
Day 1 Larvae	Random only	1611.2	3				930.4	3				531.1	3			
	Temperature only	1697.5	6	172.8	3	**$<2.2 \times 10^{-16}$**	971.4	5	81.9	2	**$<2.2 \times 10^{-16}$**	553.6	5	45.1	2	**1.62×10^{-10}**
	Temperature + Family	1738.2	34	81.3	28	**4.20×10^{-7}**	996.0	27	49.2	22	**.000752**	571.6	17	36.1	12	**0.000315**
	Temperature × Family	1762.6	85	48.8	51	0.563	1,011.4	61	30.8	34	.626	578.1	37	12.8	20	.885
Day 16 Larvae	Random only	6.157	3				48.1	3				70.2	3			
	Temperature only	235.02	5	457.7	2	**$<2.2 \times 10^{-16}$**	188.9	4	281.5	1	**$<2.2 \times 10^{-16}$**	242.8	5	345.2	2	**$<2.2 \times 10^{-16}$**
	Temperature + Family	247.15	23	24.3	18	.147	203.4	23	29.1	19	.0647	245.9	11	6.2	6	0.401
	Temperature × Family	256.74	53	19.2	30	.936	220.0	42	33.1	19	**.0232**	259.8	23	27.8	12	**.00584**
Pupae	Random only	214.81	3				582.2	3				281.7	3			
	Temperature only	219.92	4	10.2	1	**.00138**	583.9	4	3.5	1	.0601	288.3	5	13.1	2	**.00143**
	Temperature + Family	229.93	12	20.0	8	**.0103**	594.9	23	22.0	19	.285	293.6	12	10.6	7	.158
	Temperature × Family	235.3	20	10.7	8	.217	601.8	42	13.7	19	.801	303.3	25	19.5	13	0.109
Adults	Random only	48.107	3				185.3	3				144.6	3			
	Temperature only	54.505	4	12.8	1	**.000347**	185.6	4	0.5	1	.471	148.5	4	7.9	1	**.00486**
	Temperature + Family	58.141	7	7.1	3	.0692	190.4	9	9.7	5	.085	150.7	10	4.3	6	.631
	Temperature × Family	59.719	10	3.2	3	.368	191.2	14	1.6	5	.898	151.7	16	1.9	6	.928
Mass (g)																
Pupae	Random only	815.84	3				1829.9	3				891.6	3			
	Temperature only	824.33	4	17.0	1	**3.78×10^{-5}**	1829.9	4	0.1	1	.815	904.6	5	26.0	2	**2.29×10^{-6}**
	Temperature + Family	834.91	12	21.2	8	**.00676**	1847.7	23	35.5	19	**.0121**	907.0	12	4.9	7	.668
	Temperature × Family	838.04	20	6.3	8	.618	1856.7	42	18.1	19	.519	913.2	25	12.3	13	0.503
Adults	Random only	239.91	3				621.9	3				612.1	3			
	Temperature only	243.38	4	6.9	1	**.00846**	622.1	4	0.4	1	.533	620.6	4	16.9	1	**3.94×10^{-5}**
	Temperature + Family	247.51	7	8.3	3	**.0409**	626.2	9	8.1	5	.152	623.9	10	6.7	6	.345
	Temperature × Family	248.25	10	1.5	3	.69	628.3	14	4.3	5	.51	625.7	16	3.6	6	.733
Development time (days)																
Larvae	Random only	−577.93	3				−1387.0	3				−1065.7	3			
	Temperature only	−520.52	4	114.8	1	**$<2.2 \times 10^{-16}$**	−1223.6	4	326.7	1	**$<2.2 \times 10^{-16}$**	−830.7	5	470.1	2	**$<2.2 \times 10^{-16}$**
	Temperature + Family	−514.1	12	12.8	8	.118	−1197.5	23	52.2	19	**6.10×10^{-5}**	−822.7	12	15.9	7	**.0257**
	Temperature × Family	−509.57	20	9.1	8	.337	−1185.9	42	23.2	19	.229	−807.9	25	29.6	13	**.00542**

(Continues)

TABLE 3 (Continued)

Trait	Factor	*C.* aff. *dilaticollis*					*C.* aff. *dorsalis*					*C. placida*				
		LL	*df*	χ^2	χ^2 *df*	*p*	LL	*df*	χ^2	χ^2 *df*	*p*	LL	*df*	χ^2	χ^2 *df*	*p*
Pupae	Random only	−139.62	3				−380.4	3				−399.8	3			
	Temperature only	−105.05	4	69.1	1	**$<2.2 \times 10^{-16}$**	−294.0	4	172.6	1	**$<2.2 \times 10^{-16}$**	−298.0	4	203.5	1	**$<2.2 \times 10^{-16}$**
	Temperature + Family	−103.33	7	3.5	3	.327	−292.4	9	3.3	5	.652	−292.4	10	11.2	6	.0812
	Temperature × Family	−103.1	10	0.4	3	.931	−286.9	14	11.0	5	.0511	−290.6	16	3.7	6	.72

Note: For all models that include fixed effects, the statistics for a log-likelihood ratio test comparing it to the preceding model are reported. A significant *p*-value means that the model is significantly better than the preceding one.
Abbreviation: LL, log-likelihood.
p values in bold are significant ($p < .05$).

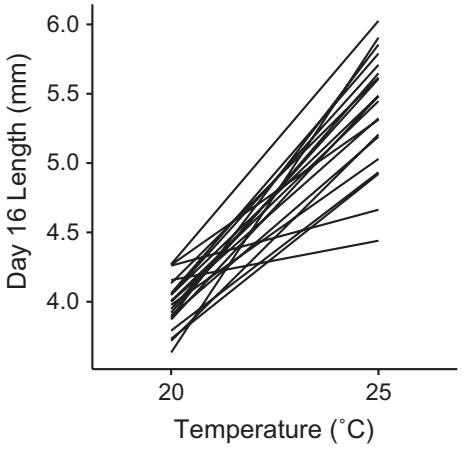

FIGURE 3 *Cephaloleia* aff. *dorsalis* reaction norms for larval size at different temperatures. Each line represents the mean performance of a genetic family in an environment (sample size and statistical analyses for genotype × environment interactions are included in Table 3, $p < .05$)

insects range from 0°C to ~45°C (Hoffmann et al., 2013; Sunday et al., 2011).

In a previous study, we used similar methodologies to those applied by global analyses of thermal tolerance (García-Robledo et al., 2016). We determined that *Cephaloleia* beetles lose motor control when exposed to temperatures between 40 and 45°C (García-Robledo et al., 2016). Predicting local extinctions based on tolerance to extreme temperatures is unrealistic, as population decline will occur before reaching such high temperatures. In a recent analysis, we determined that *Cephaloleia* beetles are already operating at their upper thermal limits for larval survival, adult longevity and fecundity. With a temperature increase of just 2°C, populations of *Cephaloleia* beetle will decline, accelerating lowland demographic attritions (García-Robledo & Baer, 2021).

Laboratory experiments using constant temperatures, such as those described in this study are relevant to understand organismal responses in more realistic situations, for example when insects experience fluctuating temperatures. Previous studies suggest that insect growth and longevity are not affected by small temperature fluctuations. For example, cohorts of the yellow dung flies (*Scatophaga stercoraria*) raised at constant temperatures, or at temperatures fluctuating ±3°C display similar growth rates and longevity (Kjærsgaard et al., 2013). However, when increasing the range at which temperature fluctuates, the general result is that insect performance declines (Kjærsgaard et al., 2013). This suggests that fluctuating temperatures may represent more challenging environments than constant temperatures, and our study potentially underestimates deleterious effects of temperature increase on insect population dynamics (Auad et al., 2015; Kjærsgaard et al., 2013).

We found more positive than negative correlations across temperatures. These results seem to support a 'winner and loser genotypes' scenario over the jack-of-all-trades trade-offs hypothesis (Agrawal, 2020; Palaima & Spitze, 2004). However, we must be cautious regarding this overall conclusion. Because of high mortality at

extreme temperatures, there were many genetic correlations that we could not estimate. For *C. placida*, the beetle species able to survive at colder temperatures, we detected negative covariance in larval growth, suggesting that there are cold- and hot-tolerant genotypes in the population. For the other two beetle species, we were not able to test potential trade-offs at cold temperatures because individuals reared at 15°C died more often than siblings reared in 20°C or 25°C. Similarly, we could not calculate any genetic correlations with lethal temperatures (10°C, 30°C or 35°C). One possibility is that such trade-offs exist, but strong selection and high mortality at extreme temperatures make challenging to achieve the sample size required to detect negative genetic correlations. An alternative is that as temperatures increase, some genotypes will be winner and others will be losers to climate change.

How common is our finding that tropical insects are thermal specialists due to heritable performance trade-offs at extreme temperatures? Unfortunately, the heritability of thermal responses has primarily been studied in model insects such as fruit flies (Karan et al., 1999, references in Hoffmann et al., 2003, van Heerwaarden et al., 2016, Mitchell & Hoffmann, 2010). Only a few studies have investigated tropical species or populations (van Heerwaarden et al., 2016; Karan et al., 1999; Mitchell & Hoffmann, 2010). In

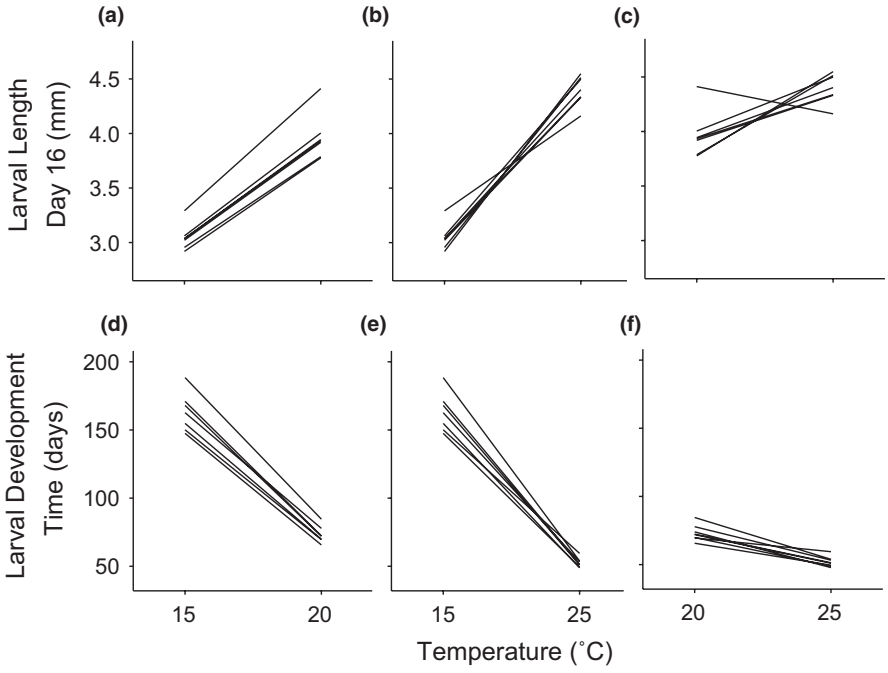

FIGURE 4 *Cephaloleia placida* reaction norms for larval size and time to pupation at different temperatures. Each line represents the mean performance of a genetic family in an environment. Lengths of two-week-old larvae: (a) 15°C versus 20°C, (b) 15°C versus 25°C, (c) 20°C versus 25°C. Time to pupation: (d) 15°C versus 20°C, (e) 15°C versus 25°C, (f) 20°C versus 25°C. (Sample size and statistical analyses for genotype × environment interactions are included in Table 3, $p < .05$.)

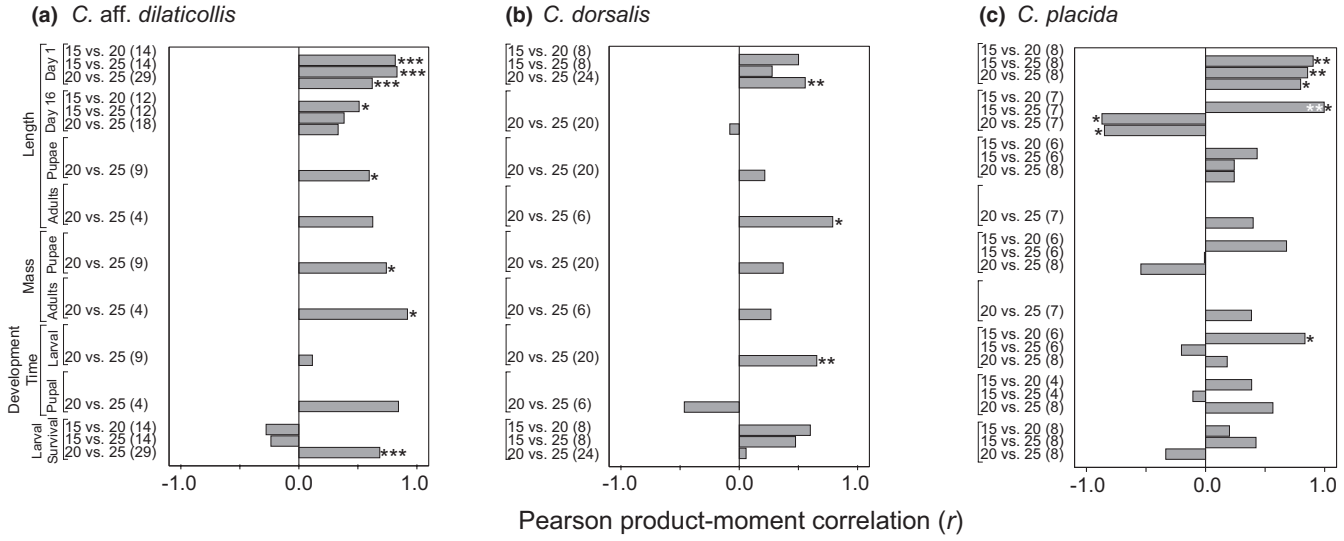

FIGURE 5 Summary of pairwise correlations for developmental traits and larval survival of genetic families reared at multiple temperatures. Sample size (number of families) is given in parentheses for each correlation. (a) *Cephaloleia* aff. *dilaticollis*, (b) *C.* aff. *dorsalis* and (c) *C. placida*. Asterisks indicate the significance of the Pearson's product–moment coefficient: $^{*}p < .10$, $^{**}p < .01$, $^{***}p < .001$

these studies, even the tropical flies were viable over ranges at least 10°C wide and many species had thermal ranges 15–20°C wide. Additionally, focal populations did not display heritable thermal responses. Those populations showing heritable responses, genotypes displayed positive genetic correlations. Because few studies included lethal and sub-lethal temperatures, the variation in the *Drosophila* studies could be an artefact of sampling different portions of populations' viable ranges (Hoffmann, 2010). Our results highlight the importance of studying more diverse taxa over their entire temperature ranges to determine whether there are uniform patterns of heritability for thermal performance.

Currently, La Selva *Cephaloleia* populations cannot survive at 30°C, which approximates the mean temperature predicted for the year 2100 (Clark et al., 2015). The time required for *Cephaloleia* to produce at least one viable adult (i.e. generation time) is between 12 and 28 weeks (García-Robledo & Horvitz, 2011). This suggests that *Cephaloleia* beetles must adapt in less than 149–348 generations in the next 80 years. Even for insects with short generation time, such as fruit flies (*Drosophila melanogaster*), rapid adaptation beyond current thermal limits seems unlikely (Gilchrist et al., 1997). Individuals of the harlequin fly (*Chironomus riparius*, Chironomidae) exposed to high temperatures (i.e. 26°) display a reduction in larval mortality of 12% after only three to five generations. These results suggest that some insect populations already harbour the genetic variation required to cope with future extreme temperatures (Foucault et al., 2018). Predictions based on phylogenetic analyses of thermal tolerance are not that optimistic. Thermal limits of ectotherms seem to evolve at a rate of 0.8°C every million years (Bennett et al., 2021). It is uncertain whether *Cephaloleia* populations will have enough time to adapt to the fast pace of global warming.

Although our results show that larval performance at different temperatures is heritable, we cannot conclude that *Cephaloleia* populations harbour the genetic variation required to adapt to novel temperatures. The main shortcoming of our heritability estimates is that they include both additive and nonadditive genetic variation. It is also possible that strong differences among genotypes are the result of maternal effects (Mousseau & Dingle, 1991). Because mothers were collected in the wild, larval performance may be the result of differences in maternal initial physiological condition (Mousseau & Dingle, 1991). Determining the relative effects of maternal effects on thermal responses is challenging, as maternal effects may affect physiological responses, even in highly heritable traits (Jenkins & Hoffmann, 1994). An additional challenge to disentangle the effects of additive and nonadditive genetic variation is that paternal and maternal effects may have opposite effects on thermal tolerance (Crill et al., 1996).

For most developmental traits, genetic variation within populations was usually reduced at extreme temperatures. This suggests that even when combining additive and nonadditive genetic variation, performance of all genotypes is equally reduced in challenging environments. Genetic variation among populations might play a larger role than intra-populational variation when facing novel temperatures. Populations of widespread temperate insects are adapted to local temperatures (Günter et al., 2020). Although individuals within a population display similar responses to increasing temperatures, differences in phenotypic plasticity among populations ensure that some individuals are preadapted to warmer conditions (Günter et al., 2020). As shown in our study, such plastic responses might not be possible for tropical insects, which experience more constant temperatures during their lifetime (Sunday et al., 2011, 2014).

Our results suggest that many tropical ectotherm populations will be unable to adapt to human-induced climate change rapidly enough to persist in situ (Van der Putten et al., 2010). Narrow thermal specialization will make it very difficult for populations to persist in their current locations as temperatures rise. We found that an increase from 25°C to 30°C caused 100% juvenile mortality in our study populations. The fact that many traits were suboptimal at current average temperatures is also concerning, as it suggests that local adaptation is already lagging behind current temperatures. The combination of thermal specialization and lagging adaptation means that many tropical ectotherms will experience, or may already be experiencing, lowland attrition (Brusch et al., 2016; Nowakowski et al., 2017; Whitfield et al., 2007). Specialized tropical ectotherms could remain in their thermal ranges by migrating up mountains, but such migrations are limited by other abiotic and biotic factors, as well as species' dispersal capabilities (Corlett, 2011). Tropical species occurring in areas without mountains, such as most of the lowland Amazonia, will have nowhere to move to. The consequences of a scenario of CO_2 emissions 'business as usual' (temperature increase of 4–5°C by 2100, Masson-Delmotte et al., 2018) would be devastating. However, our results also suggest that if humans can limit or slow warming, many tropical ectotherms might persist in their current locations and potentially adapt to warmer temperatures.

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PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.9kd51c5hb

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SUPPORTING INFORMATION

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