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Functional Ecology

Environmental predictability drives adaptive within- and transgenerational plasticity of heat tolerance across life stages and climatic regions

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Author contributions

FD and LMM conceived the idea and designed laboratory experiments. FD, NT and JMC performed all laboratory experiments. FD conducted all statistical analyses of phenotypic data. BK and RBH designed all simulation modelling and computational work. BK conducted computational work and analyzed simulated data. FD, NT, JMC, BK, RBH and LMM were all involved in the analysis and writing of the manuscript.

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Abstract

1. Although environmental variability and predictability have been proposed as the underlying ecological context in which transgenerational plasticity (*TGP*) arises, the adaptive significance and interaction with within-generation plasticity (*WGP*) in such scenarios is still poorly understood. In order to investigate these questions, we considered the tolerance to upper thermal limits of larvae and adults of the desert endemic *Drosophila mojavensis* adapted to different climatic regions (Desert vs Mediterranean climate).
2. Thermal plasticity was investigated by acclimating parents and offspring at 36°C (versus at 25°C). We then used historical temperature variation data from both regions to perform individual-based simulations by modeling expected components of adaptive plasticity in multiple life stages.

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3. Our results indicated that thermal response to ramping heat shocks was more pronounced in larvae, where acclimation treatments in parents and offspring increased their heat-shock performance, while heat knockdown in adults was only increased by offspring acclimation of adults. The relative contribution of *WGP* and *TGP* was greater for the population from the more thermally variable Sonoran Desert.
4. Similarly, individual-based simulations of evolving maternal effects indicated that variation in tolerance to upper thermal limits across life stages and climates is expected from its adaptive significance in response to environmental predictability.
5. Our approach offers a new perspective and interpretation of adaptive plasticity, demonstrating that environmental predictability can drive thermal responses across generations and life stages in a scenario with regional climate variability.

Key words: Within/transgenerational plasticity, acclimation, carry-over effects, heat-shock tolerance, individual-based simulations, *Drosophila mojavensis*.

Introduction

The role of the environment in shaping phenotypic variation has been recognized since the very beginning of the genotype vs environment discussion (Baldwin 1896). The importance of these dynamics has led to the view that an organism's phenotype is the result of a unique interaction between its genotype and its whole temporal trajectory of external environments (Fusco and Minelli 2010). Although genetic variation was initially considered the ultimate source of change, non-genetic inherited changes such as maternal effects have been well recognized as a source of phenotype variation for decades (Kirkpatrick and Lande 1989; Nelson and Nadeau 2010; Moore et al. 2019). These sources of transgenerational variation were traditionally treated as troublesome, unwanted effects masking the genetic variation, so much so that experiments were designed in order to remove them (Falconer 1981). The reconsideration of these effects has illustrated how the parental environment can contribute to the phenotype of the next generation, acting as a transgenerational form of phenotypic plasticity (Heard and Martienssen 2014). Currently, it is well recognized that parents can alter the phenotype of their offspring through a number of non-genetic or epigenetic processes (Nestler 2016), such as DNA methylation

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(Arsenault et al. 2018), mRNA (Ahi et al. 2018), transposons (Migicovsky et al. 2014) or small RNAs (Stief et al. 2014).

There is increasing evidence demonstrating the role played by the carry-over effects of environmental exposure across different time scales over a single generation (Nelson and Nadeau 2010). The genetic basis of within-generation plasticity (*WGP*) and its role in buffering or favoring natural selection via genetic assimilation has been extensively explored (Pigliucci et al. 2006; Badyaev 2009). Ecological conditions in which natural selection can influence the level of an organism's response to environmental fluctuations leading to adaptive *WGP* have been reported in many taxa (Via 1993; Delpuech et al. 1995; Moreteau et al. 2003; Crispo 2008; Lind et al. 2011). This evidence has established a solid theory including both empirical and substantial theoretical modelling (Jong 1995; Lande 2009; Chevin et al. 2010; Herron and Doebeli 2011), defining the interaction between selection and *WGP* (Schlichting and Pigliucci 1998; Pigliucci et al. 2006; Fusco and Minelli 2010).

On the other hand, the role transgenerational plasticity (*TGP*) in evolution is less understood. Most of the effort has been focused on demonstrating transmissible effects over generations, which has been corroborated for many traits (Yin et al. 2019), as well as its associated molecular mechanisms (Nelson and Nadeau 2010; Heard and Martienssen 2014; Nestler 2016). These transgenerational effects are currently lacking a unified definition, being currently referred to through numerous different terms such as non-genetic inheritance, maternal effects, anticipatory parental effects, carry-over effects, intergenerational effects, among others (Nelson and Nadeau 2010; Heard and Martienssen 2014; Donelson et al. 2018). Here we focus on a definition that allows the study of whether such responses are adaptive as opposed to merely carry-over effects: as reviewed by Donelson et al. (2018), we consider *TGP* to describe the effect of interactions between environmental conditions experienced by parental and offspring generations on the offspring phenotype. This definition is in line with that of traditional maternal (or paternal) effects and their role in adaptation (Mousseau and Fox 1998; Newcombe et al. 2015; Proulx and Teotónio 2017; Moore et al. 2019), and allows for predictions as to how the parental environment can influence offspring performance (Donelson et al. 2018).

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Given the potential of *TGP* to contribute to the rapid adaptation of populations to a changing global climate (Hoffmann and Sgró 2011; Sgrò et al. 2016; Donelson et al. 2018; Bonamour et al. 2019), *TGP* is considered as a potential source of ecologically and evolutionarily meaningful variation (Burgess and Marshall 2011; Herman and Sultan 2011; Bonduriansky et al. 2012). Predicted climate change has inspired a multitude of studies demonstrating the role of acclimation (Anderson et al. 2012) in enabling organisms to overcome periods of environmental change within a single generation (Hoffmann and Sgró 2011; Overgaard et al. 2011). Since such changes can persist across multiple generations, adaptive *TGP* has been proposed as an important mechanism to overcome stress environments in a number of species, including plants (Herman and Sultan 2011; Münzbergová and Hadincová 2017), nematodes (Massamba-N'Siala et al. 2014; Webster et al. 2018), vertebrates (Badyaev 2009; Steenwyk et al. 2018), marine species (Guillaume et al. 2016; Ryu et al. 2018) and insects (Schiffer et al. 2013; Zizzari and Ellers 2014). The role of these plastic responses is commonly assumed to be similar to what has been found for *WGP*, buffering populations against extreme fluctuations in the near term or canalizing natural selection in the long term (Münzbergová and Hadincová 2017). However, theoretical considerations (Badyaev and Uller 2009; Sheriff et al. 2018) supported by theoretical models (Kuijper and Hoyle 2015; Proulx and Teotónio 2017) have pointed to environmental variability and predictability across generations as the evolutionary scenario that promotes adaptive *TGP* over and above *WGP*.

With a few exceptions (Badyaev and Oh 2008; Burgess and Marshall 2011), historical environmental variation is often ignored when defining ecologically relevant cues to trigger *TGP* in the lab (Donelson et al. 2018). Regular and predictable environmental fluctuations such as seasonality offer a potential scenario that facilitates parental-offspring environment predictability (Marshall and Burgess 2014), since the level of autocorrelation across the life cycle has been considered a determinant for adaptive *TGP*. Indeed, recent reviews have pointed to *match*/*mismatch* experiments from factorial designs in which both parents and offspring are exposed to alternative environments (often stress and non-stress) as an indication of predictability and therefore adaptive *TGP* (Sheriff et al., 2018; Uller et al., 2013). The impact of predictability resulting from *matched*, when compared to *mismatched* cues, is suggested from the costs of *TGP* when the parental environment does not efficiently predict that in the offspring

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(*mismatched* cues). However, this approach has been argued as insufficient when disentangling adaptive *TGP* from other non-predictive carry-over effects such as *silver spoons* (where individuals that develop in good conditions experience fitness benefits as adults) in certain conditions (Engqvist and Reinhold 2016), which again has left several questions regarding the interplay between *WGP* and *TGP* unresolved: Do they respond to the same kind of fluctuations? Are they convergent responses to fluctuations? What is their relative importance in a given ecological context?

Here we propose to combine experimental evidence from *match*/*mismatch* experimental framework (Uller et al. 2013; Sheriff et al. 2018) where parents and offspring are both exposed to either moderate or stress temperatures, with individual-based simulations data for the evolution of *WGP* and *TGP* (Kuijper and Hoyle 2015), to investigate the adaptive component of plasticity of heat tolerance in two genetically and ecologically distinct populations of the desert *Drosophila mojavensis* (Heed 1978; Matzkin 2014). The central hypothesis is that evolution under a more fluctuating environment (Sonoran Desert relative to buffered Mediterranean climate of Santa Catalina Island, California) will exhibit higher thermal plasticity under *matching* environments between parents and offspring, while minimizing unpredictive carry-over effects under *mismatched* acclimation treatments (Uller et al. 2013; Engqvist and Reinhold 2016; Sheriff et al. 2018). We adapted the simulation model to the particular ecological conditions of *D. mojavensis* using historical climate data from the sampled regions in order to generate predictions for adaptive responses in larvae and adults. Our results point to adaptive differentiation in thermal plasticity linked to environmental predictability across life stages in an ecological context with substantial regional climate variability.

Materials and methods

Samples

Each experimental population was established by pooling four isofemale lines of *D. mojavensis* originally collected in Santa Catalina Island, California or Sonoran Desert, Mexico (hereafter, Catalina and Sonora) (Figure 1a). Whereas the population from the Sonoran Desert experiences higher temperatures (mean and maximum) and variance (diurnal and annual) relative to that from

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Mediterranean climate in Catalina Island (Figure 1b). The established mass-bred populations were reared at 25°C, under 12:12 h light:dark cycle and controlled density conditions in 8-dram glass vials with banana-molasses media for four generations before experiments (Coleman et al. 2018). Since *D. mojavensis* females multiply mate (Knowles and Markow 2001), each of the founder isofemale lines per population will tend to be segregating variation from multiple sires. Hence at minimum, each of the populations captured variation from at least 16 independent segregating haploid genomes, but likely more depending on how often the female mate, which we considered enough for interpopulation comparisons. A more expanded sampling will be necessary in future studies for deep intrapopulation genetic analyses and mapping.

Experimental design

Heat-shock tolerance was assessed in response to previous acclimation exposure performed in parents and offspring at either moderate or stress temperatures of 25°C and 36 °C respectively. The experiment had a factorial design with two parental treatments (25°C and 36°C in 10-12 days-old adults) and two offspring treatments (25°C and 36°C in larvae and adults) for each population (Figure 1c). The parental generation of both populations was divided into two cages with a banana-molasses food plate and each cage was subjected to either 25 or 36°C treatments in a Percival incubator for 24 h prior to oviposition. Following this 24 h acclimation period, a new food plate was placed in each cage for flies to oviposit at 25°C for another 24 h and these plates were then divided into two equal parts. Each half-plate containing F_1 eggs was placed at either 25°C or 36°C for 36 h. The prolonged acclimation period for larvae with respect to that in adults was used in order to account for the different thermal limits between life stages. Larvae are much more resistant to heat shocks (see results) and therefore required prolonged time to trigger heat-shock responses. The chosen temperature and periods correspond to the maximum treatment that trigger a heat-shock response without killing individuals in the process. Hatched first instar larvae were then placed in groups of 30 into food vials. Approximately 40 vials per each of the 8 half-plates representing the different combinations of parental and F_1 larval treatments were collected. Half of these vials were immediately used to test for the heat-shock tolerance of first instar larvae. The second half of these vials were maintained at 25°C until flies eclosed to perform experiments on adults.

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To test for possible interactions between parental, F_1 larval and F_1 adult heat acclimation, the above eclosed adults from the 8 parental/F_1 larval combinations were split one more time. When the F_1 adults were approximately 10 days of age, half of them were subjected to either 36°C or 25°C treatments for 24 h. The next day, males and females from the 16 treatments were tested for heat-shock tolerance.

Heat-shock experiments

Thermal performance of first instar larvae and adults was assessed using a ramping treatment in a water bath with temperature controlled by a Thermo Scientific Circulator (AC 200). The ramping treatment was set between 30°C up to 40°C. First, temperature was held at 30°C for 15 min and then it was increased by 0.13°C/min until reaching 40°C, where temperature remained constant for the rest of the experiment depending on the fly stage in test (see below). The ramping rate was estimated from field measurements of rotting cacti in Organ Pipe National Monument (Arizona, USA) during summertime (Authors' unpublished data).

For larvae, vials with food containing groups of 30 larvae were submerged in the water bath for a post ramping period of 1.5 h and 2 h at 40°C. Post ramping periods were selected based on preliminary data in order to capture mid and high stressful treatments and correspond to the *HS* term in the linear model (see statistical analysis below). For the larval assays, the number and time of pupation and hatched adults was recorded on a daily basis for 10-12 replicates per treatment. For adult performance, males and females were placed in individual 1-dram capped vials, then randomly arranged on clamps on an acrylic frame and submerged in a transparent water bath allowing the visual inspection of the vials. All flies were constantly observed and scored for time until heat knockdown was reached. Knockdown was defined as the moment in which flies were not able to hold themselves upright or move after being stimulated by a strong flashlight. A total of 15 replicates were scored per treatment combination of acclimation performed in parents, F_1 larvae and adults (16 combinations).

Statistical analysis and modelling

Acclimation effects for larvae and adults were tested using a generalized linear model (*GLM*). These models evaluated *WGP* and *TGP* as a result of acclimation in parents and offspring as well

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as additional effects specific to each stage. In the case of larval traits, heat tolerance included heat-shock period:

$$y = \mu + \left(Pop + Accl_{parents} + Accl_{larva} + HS\right)^4,$$

where *y* is the thermal tolerance (viability or development time components larva-pupa-adult), μ is the mean thermal tolerance, Pop is the population effect (Sonora vs Catalina), $Accl_{parents}$ is the acclimation effect performed in parental generation and therefore represents *TGP*, while $Accl_{larva}$ is the *WGP* effect of acclimation of F_1 larva, and HS is the post ramping heat-shock period performed in larva (1.5 or 2 h).

For adult traits, the model included the three instances of acclimation (parents, F_1 larvae and adults):

$$y = \mu + \left(Pop + Sex + Accl_{parents} + Accl_{larva} + Accl_{adults}\right)^5,$$

where *y*, μ, Pop, $Accl_{parents}$ and $Accl_{larva}$ are the same terms used for larval tolerance, while $Accl_{adults}$ represents the effect of acclimation performed in F_1 adults.

Viability components larva-pupa-adult were analyzed directly using a logit *GLM* link function as well as a proportion between heat-shocked larvae with respect to that of viability of non-heat-shocked samples (acclimated samples but not subjected to heat shocks) – hereafter standardized viability. Because standardized viability does not follow a binomial distribution, we used a logarithm transformation in order to fit normal distribution of data followed by a gaussian *GLM* function. Components of development time (larva-pupa-adult) as measured from heat-shocked larvae and heat knockdown in adults were analyzed through a gaussian *GLM* link function on untransformed data since data were mostly normally distributed and variances homogeneous. All these analyses were performed using the R function *glm*. Specific comparisons were performed using a Tukey post-ANOVA through the R package *multcomp*.

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Variation partitioning analysis

Fitted models were also used to perform a variation partitioning analysis (Borcard et al. 1992) to assess the relative contribution of *WGP* and *TGP* in each climate region. For this, fitted models were run by population, heat-shock periods (larval data) and sex (adult data). Each acclimation effect was fitted independently as well as combined, and then coefficients of determination were extracted to estimate their relative contribution to total variation using the function *varPart* of R package *modEvA* (Barbosa et al. 2013, 2016).

Individual based simulations of WGP and TGP

We used individual-based computer simulations to assess how differences in climatic conditions between Sonora and Catalina affect the long-term evolution of within and transgenerational plasticity (see Appendix S1 in Supporting Information for a more extensive description of parameter values included in the model, and Appendix S2 for analysis of the adaptation of the temperature time series from historical temperature data). Extending previous quantitative genetics models on cascading maternal effects (Kirkpatrick and Lande 1989; Kuijper and Hoyle 2015), we consider a well-mixed population of N = 10,000 diploid individuals with non-overlapping generations. Individuals are then allowed to adapt to a realistic fluctuating environment as extracted from historical climate data from Catalina and Sonora [Data provided by National Centers for Environmental Information, National Oceanic and Atmospheric Administration (NOAA) from their web site https://www.ncdc.noaa.gov/cdo-web/datasets#NORMAL_HLY (Figure 1)], during 50,000 generations (see Figure S6 for an example simulation), where within and between generational plasticity is allowed to vary between larval and adult individuals. Hence, the phenotype of a larval individual is z_{lv} while the adult phenotype is z_{ad}. Specifically, the larval phenotype $z_{\mathrm{lv},t+\tau_0}$ in generation t at the time of birth τ_0 (where $\tau_i = \frac{i}{\ell}$ is the number of days relative to the total lifespan ℓ measured in days) is given by

$$z_{\mathrm{lv},t+\tau_0} = a_{t+\tau_0} + b_{\mathrm{lv},t+\tau_0}\varepsilon_{t+\tau_0} + m_{\mathrm{lv},t+\tau_0}z^{*}_{\mathrm{ad},t-1} + e_{t+\tau_0}. \quad (1)$$

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Here, the larval phenotype $z_{\mathrm{lv},t+\tau_0}$ is affected by three evolving traits, with $a_{t+\tau_0}$ reflecting the genetic basis of the phenotype in the absence of within and transgenerational plasticity, $b_{\mathrm{lv},t+\tau_0}$ reflecting the strength of larval within-generational plasticity in response to the environment experienced at the time of birth $\varepsilon_{t+\tau_0}$ and finally $m_{\mathrm{lv},t+\tau_0}$ reflects the strength of the transgenerational effect that depends on the adult mother's phenotype $z^*_{\mathrm{ad},t-1}$, where the * denotes a phenotype after it experienced survival selection. The variable $e_{t+\tau_0}$ reflects developmental noise, which is a random variable drawn from a normal distribution with mean 0 and variance σ_e^2.

After birth, a larva with phenotype z, plasticity b and maternal effect m experiences stabilizing mortality selection at every day of its life. Its survival probability $s_{t+\tau_i}(z,b,m)$ at generation t and day $\tau_i\ell$ is given by

$$s_{t+\tau_i}(z,b,m) = s_{\min} + (1 - s_{\min})\exp\left\{-\frac{1}{2}\left[\frac{\left(z-\varepsilon_{t+\tau_i}\right)^2}{\omega_z^2} + \frac{b^2}{\omega_b^2} + \frac{m^2}{\omega_m^2}\right]\right\}, \quad (2)$$

where $s_{\min}$ is a baseline survival probability to prevent populations going extinct (as we are interested in the values of m and b that evolve in certain regimes rather than in where and when populations go extinct). Throughout, we assume $s_{\min} = 0.5$. Within the exponential term, we assume that the optimal phenotype (to maximise survival probability) is $\varepsilon_{t+\tau_i}$, the temperature of that day (see Appendix S2 "Adaptation to temperature timeseries"), while ω_z^2 is the width of the selection function, small (large) values of which imply strong (weak) selection. Next, the terms $\frac{b^2}{\omega_b^2}$ and $\frac{m^2}{\omega_m^2}$ reflect stabilizing selection against within generational plasticity and maternal effects respectively (Kuijper and Hoyle 2015).

Larvae which have survived according to eq. (2) for $\tau_{\mathrm{ad}}\ell$ days become adults, after which they develop an adult phenotype $z_{\mathrm{ad},t+\tau_{\mathrm{ad}}}$ in generation t, where within and transgenerational plasticity of the adult phenotype can evolve independently from the same traits for the larval phenotype. Hence, we have:

$$z_{\mathrm{ad},t+\tau_{\mathrm{ad}}} = a^*_{t+\tau_{\mathrm{ad}}} + b^*_{\mathrm{ad},t+\tau_{\mathrm{ad}}}\varepsilon_{t+\tau_{\mathrm{ad}}} + m^*_{\mathrm{ad},t+\tau_{\mathrm{ad}}} z^*_{\mathrm{ad},t-1} + e_{t+\tau_0}, \quad (3)$$

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where $a^*_{t+\tau_{\mathrm{ad}}}$ reflects the elevation, which is the same trait as expressed in larvae, conditional on that the individual has survived for $\tau_{\mathrm{ad}}\ell$ days (denoted by *). The strength of within-generational plasticity in adulthood is $b^*_{\mathrm{ad},t+\tau_{\mathrm{ad}}}$, which reflects the strength of the reaction norm in response to the environment $\varepsilon_{t+\tau_{\mathrm{ad}}}$ at the onset of adulthood. Regarding transgenerational plasticity, $m^*_{\mathrm{ad},t+\tau_{\mathrm{ad}}}$ reflects sensitivity to the maternal phenotype at adulthood. Here, the maternal phenotype $z^*_{\mathrm{ad},t-1}$ is the same phenotype that was experienced as larva, reflecting, for example, persistent maternally transmitted chromatin modifications, small RNAs or nutrients (Moore et al. 2019) . Finally, $e_{t+\tau_0}$ again reflects developmental noise.

The traits b_{lv}, b_{ad}, m_{lv} and m_{ad} are each assumed to be coded by single diploid loci, whereas the elevation a is assumed to be coded by 5 diploid loci, in line with previous models where the additive genetic variance in elevation is typically taken to be larger than the additive genetic variance in plasticity (e.g., Hoyle and Ezard, 2012; Lande, 2009). For the sake of simplicity, all loci are unlinked and evolve according to a continuum of alleles model (Kimura and Crow 1964). The probability that each allele mutates per generation is $\mu = 0.01$, after which a random number drawn from a normal distribution with mean 0 and variance 4×10^{-4} is added to the current allelic value.

Results

Acclimation treatments performed at 36°C (versus 25°C) in parents and F_1 larvae significantly increased tolerance of heat-shocked larvae as measured through viability components (Table 1, Figure 2a), while only within-generation acclimation increased heat knockdown in adults (Table 2, Figure 2a). Unlike viability components, development time did not always increase in response to the acclimation treatments (Table 1, Figure 2a). Larva-pupa and larva-adult components of viability and development time showed significant effects of acclimation treatments and population, whereas the percentage of hatching pupa was not affected (Table S1). Therefore, thermal responses in larva-to-pupa and larva-to-adult were highly correlated (*Viability Spearman's r* = 0.99, $P < 0.01$ and *Development time Spearman's r* = 0.94, $P < 0.01$). These

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results suggested that acclimation treatments performed in larvae only affected the larva-to-pupa transition and not pupa-to-adult.

Larval tolerance to upper thermal limits

Viability was analyzed as a response to heat-shocks following acclimation as well as standardized by the control treatments (acclimation treatments without being heat-shocked) (Table 1). Standardized viability was used to confirm whether detected responses to heat shocks persist after controlling for acclimation effects on non-heat-shocked larvae. Population, heat-shock periods, parental and F_1 larval acclimation treatments were significant for both viability and standardized viability (Table 1). Longer heat-shock periods lead to lower viability (see Figure 2a and for results at 1.5 and 2h heat shock) but tended to increase population and acclimation effects. Hereafter we focus on results obtained in for 2h heat shock in larvae (Figure 2a). All acclimation treatments increased heat tolerance, but several paired interactions were detected for viability, showing differential effects of *WGP* and *TGP* according to population, heat-shock period as well as interactions between acclimation treatments ($Accl_{larva}$*$Accl_{parents}$) (Table 1). Most of these interactions were not significant for standardized viability, except for the Pop*$Accl_{larva}$ and Pop*$Accl_{parents}$*$Accl_{larva}$ interactions (Table 1), indicating that the level of *WGP* and *TGP* were different between populations (Figure 2a). The Sonoran population exhibited the largest plastic responses, and these effects were more evident from combinations of treatments where both parents and F_1 larvae were acclimated 36°C (*matched* cues), increasing heat tolerance by up to 63% when compared to *mismatched* cues (Figure 2a). In contrast Catalina had higher plastic responses when only one of the generations was acclimated, which increased their thermal performance by up to 45% (*mismatched* cues) when compared to *matched* cues (Figure 2a).

Only population and F_1 larval acclimation affected components of development time as main effects, while the heat-shock period did not nor did any of its interactions. However, there were complex paired interactions indicating differences in the effect of parental and F_1 larval acclimation between populations as well as interactions between acclimation treatments ($Accl_{larva}$*$Accl_{parents}$) (Table 1). The triple interaction Pop*$Accl_{parents}$*$Accl_{larva}$ (Table 1) indicated a complex pattern in which Catalina exhibits positive *WGP*, but negative *TGP*, while

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the Sonoran population exhibits positive effects for both acclimation treatments (Figure 2a). Moreover, Catalina only showed *WGP* for larvae coming from untreated parents (*mismatched* cues), increasing development time by up to nearly two days, while no larval acclimation was detected as *TGP* (Table 1, Figure 2a). For the Sonoran population, the pattern was opposed to that in Catalina, both *WGP* and *TGP* were positive, increasing development time in over two days. As for viability data, these effects were much larger when both parents and F_1 larvae were acclimated at 36°C (*matched* cues) (Figure 2a).

Adult tolerance to upper thermal limits

Thermal tolerance in adults was measured as heat-knockdown time during ramping heat shocks in response to acclimation treatments performed in parents, F_1 larvae and F_1 adults. Neither the temperature experienced by parents (Table 2) nor acclimation performed in F_1 larvae affected heat knockdown in F_1 adults or any of their interactions (Table S2), so these effects were removed from the final model (Table 2). Acclimation performed in F_1 adults significantly increased heat knockdown (Table 2, Figure 2a), but the response differed between populations and sexes (Table 2, Figure 2a). Two interaction effects were detected (Table 2), suggesting that the level of acclimation performs differently between populations ($Pop{*}Accl_{adults}$) and sexes ($Accl_{adults}{*}Sex$), being higher in Sonoran females, as their heat-knockdown time increased by over 20 min, while it was increased by nearly 10 min in Catalina (Figure 2a).

Variation partitioning analysis

Relative contributions of *WGP* and *TGP* to thermal tolerance as estimated from fitted models indicated that adults not only did not express *TGP*, but had the lowest *WGP* component (14% in Sonora) when compared to that in larvae (viability = 39%, development time = 19% in Sonora) (Figure 2b). The *WGP* component of larval tolerance was higher in Sonora for both viability (39%) and development time (7%) (Figure 2b). The *TGP* component was also higher for the Sonoran population, at 17%, while it explained only 10% of variation in the population of Catalina (Figure 2b). Finally, the *TGP* component of development time explained 13% of phenotypic variation in Catalina, while the Sonoran population only exhibited 3% (Figure 2b). However, this variation in Catalina was associated with *TGP* decreasing development time in this population (Figure 2b) as opposed to Sonora.

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Individual-based simulations of within and transgenerational plasticity

Simulated values of *WGP* and *TGP* (Figures S7 and S8) were obtained for larvae and adults under different scenarios of plasticity and selection costs (see Table S3 for simulation parameters) in simulations corresponding to the same experiment as performed in the laboratory (Appendix S1), with parental and F_1 offspring environments (25 vs 36°C). Since the model does not consider direct interactions between populations and/or plastic responses, expectations for empirically detected interactions cannot be detected from plots of *match*/*mismatch* cues. Simulated data are more likely to be strictly adaptive rather than exhibit short-term carry-over effects that can generate the observed interactions (Kuijper and Hoyle 2015).

Simulated larva and adult stages evolving under a Sonoran regimen resulted in higher levels of adaptive *WGP* and *TGP* than those in Catalina (Figure 3), mimicking the main findings from the experimental evidence in all traits analyzed (Figure 2a). Viability results indeed are in line with simulated plastic responses while developmental time showed a negative *TGP* in Catalina (Figure 2a) which was not obtained from simulations (Figure 3a), but the positive value of the trait was still higher in Sonora. Adult heat knockdown tolerance supported the expectation of adaptive tolerance to upper thermal limits as observed from the simulations (Figure 3b), while there was no *TGP* in adults detected in the empirical data (Figure 2a). We found that the prediction of stronger *TGP* and *WGP* in Sonora is robust to varying the strength of fluctuating stabilizing selection (Figures S7 and S9) or varying the cost of phenotypic plasticity (Figures S8 and S10). Similarly, we find that adaptive *TGP* is generally stronger when affecting larval rather than adult traits (Figure 3 and S7, S8), again in line with empirical findings of viability and heat knockdown traits (Figure 2). Adaptive *WGP* on the other hand was expected to be higher for adult traits in simulated data (Figures S7 and S8) as opposed to empirical findings (Figure 2a), where *WGP* was clearly higher in larval traits. This result suggests additional constraints missing from our model when considering developmental stages with different reproduction costs (larval vs adult). Our model suggests that realistic fluctuations in temperature can explain the differential evolution of *TGP* and *WGP* across climatic regions.

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Discussion

By combining experimental evidence with individual-based simulations of phenotypic plasticity over generations, we were able to disentangle the adaptive significance of thermal plasticity across life stages in an ecological context with substantial climate variability in the desert *D. mojavensis*. We demonstrated that the level of variation and environmental predictability can shape tolerance to upper thermal limits within and between generations and that *TGP* evolves when the parental environment is a good predictor of that experienced by the offspring. *WGP* was higher in larvae than adults, while *TGP* was only detected in larval stages. Although both regional climates showed significant plastic responses, the population from the Sonoran Desert, evolving under high thermal variability relative to that of Mediterranean climate in Catalina Island (Figures 1b and S5) led to increased plasticity when both parents and offspring were acclimated (*matched* cues). The combined analysis of empirical and simulated data suggested that life stage and regional variation of thermal *WGP* and *TGP* is adaptive in *D. mojavensis*.

Within-generation plasticity

Acclimation performed within generations significantly increased heat tolerance in both larvae and adults, although this was only evident when acclimation was conducted in the same developmental stage, moreover acclimation treatments performed in larvae did not affect tolerance in adults. As expected from a costly temporal response (Krebs and Loeschcke 1994; Dahlhoff and Rank 2007), this result demonstrates that acclimation, as performed through a brief exposure to an environmental cue, does not provide hardening against subsequent heat-shocks occurring in the long term. However, this acclimation still affected later larval stages, as evident from the pronounced effect that acclimated larvae had on development time. Changes detected in development time are likely a consequence of the cost associated with the heat shock response in each population. This acclimation effect commonly known as heat hardening, has been widely detected across several species for decades (Hoffmann et al. 2003; Sgrò et al. 2010; Kellermann and Sgrò 2018), even in *D. mojavensis* (Krebs 1999; Krebs and Bettencourt 1999). Heat hardening is mainly caused by rapid expression of heat-shock proteins (HSPs) and other molecular components that protect denatured proteins and tissues from damage caused by high thermal exposures (Dahlgaard et al. 1998; Bahrndorff et al. 2010; Diaz et al. 2015; Cai et al.

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2017). These components are known to accumulate rapidly during mid-range temperatures (*e.g.* 36°C) as occurs in *D. mojavensis* (Krebs 1999; Krebs and Bettencourt 1999).

We observed that *WGP* had a higher contribution to larval tolerance when compared to adult tolerance based on variation partitioning. This is consistent with literature on thermal tolerance in several organisms, reporting a greater thermal resistance at early life stages when compared to adults (Sørensen and Loeschcke 2002; Zizzari and Ellers 2014). Early stages including larva, are more bound to the fluctuations of their environment since they are constrained to their substrate, while flying adults can seek more suitable thermal microclimates (Krebs and Loeschcke 1995; Feder et al. 1997). Moreover, the molecular machinery of heat-shock response is known to involve considerable energy cost (Krebs and Loeschcke 1994; Dahlhoff and Rank 2007), which often leads to trade-offs between life stages and reproductive-related behaviors (Jørgensen et al. 2006; Zhang et al. 2015) leading to more limited *WGP* in adults (Sørensen and Loeschcke 2002) as has been previously found in *D. mojavensis* (Patton et al. 2001; Fasolo and Krebs 2004).

Transgenerational plasticity

We detected *TGP* only for larval tolerance, where acclimated parents led to larvae that were more resistant to upper thermal limits. The parental acclimation had an opposed effect on development time of Catalina vs Sonora, increasing development time in Sonora but decreasing in Catalina. This result suggests potential costs on development associated with *TGP* in Sonora and supports the major role of plastic responses in early stages discussed above for *WGP*. Unlike *WGP*, inferring the adaptive significance of *TGP* is more challenging. Despite the recent interest in non-genetically inherited effects and their role in evolution (Mousseau and Fox 1998; Galloway and Etterson 2007; Bonduriansky et al. 2012; Nestler 2016), more particularly for climate change scenarios (Burgess and Marshall 2011; Münzbergová and Hadincová 2017; Bonamour et al. 2019), little attention has been paid to formally testing their adaptive significance. As suggested by Donelson et al. (2018) and Uller et al. (2013), these effects are often negative, neutral (Sikkink et al. 2014) or comparatively much weaker than *WGP*. The observed positive *TGP* could still be a simple non-adaptive carry-over effect, a consequence of stressed embryos during parental acclimation or a *silver spoon* effect (Engqvist and Reinhold 2016; Sheriff et al. 2018). A more formal link to the adaptive significance of these effects should

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be investigated in relation to the predictability of environmental variation while accounting for the life cycle of the target species (Bonamour et al. 2019). Based on this premise, we investigated the effect of parent-offspring predictability of climatic variation over time on the evolution of simulated *TGP* and *WGP* in a realistic environment (Figures S6-S10). Our simulated data indicated that *TGP* on larval traits is stronger because the parental phenotype is more likely to predict the environment experienced by its offspring during their larval stage, which strongly suggest that *TGP* is likely to be adaptive in larvae. The environment is more likely to have changed when offspring are adults.

Surprisingly, although to a lesser extent, our simulations also predicted *TGP* for adults. The absence of *TGP* in our empirical adult data as opposed to simulated data suggests that the brief environmental cue used to treat parents may not be strong enough to trigger a plastic response between adult generations. However, the parent-offspring predictability included in the simulated data suggests potential effects for longer cues, such as for example when individuals are exposed to environmental cues during a great part of or whole life cycle, a prediction that remains to be formally tested. Qualitative differences between larvae and adults are also expected from the major role played by maternal molecular factors in early stages before hatching larva (Tadros and Lipshitz 2009). This is more related to the limited transcriptional capacity of *Drosophila* embryos as for other oviparous ectotherms, being highly dependent on maternal factors in comparison to later stages, which makes them particularly sensitive to thermal exposure (Walter et al. 1990). Maternal oogenesis establishes the early embryonic transcriptome and proteome (Schüpbach and Wieschaus 1986; Wieschaus 1996; Tadros and Lipshitz 2009), which are therefore major determinates of embryo fitness. Recently Lockwood et al. (2017) have found molecular evidence that demonstrates a positive effect of small heat-shock proteins from maternal ovaries on the thermal performance of embryos in *D. melanogaster*. This fact offers an additional selection pressure for maternal effects on early stages, particularly for recently hatched larvae that can potentially carry over a great load of these maternal factors.

Adaptive significance of WGP and TGP is related to regional climate

The environment of the Sonoran Desert exhibits more climatic variability compared to the Mediterranean and buffered climate of Catalina Island and was therefore predicted to express

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higher plastic responses (Figures 1b and S5). Except for adult data (*TGP* not detected for heat knockdown), all traits analyzed exhibited regional variation. For larval tolerance, variation partitioning analysis evidenced greater relative components of *WGP* and *TGP* in the Sonoran region when compared to those in Catalina. Overall, this result agreed with our expectations of adaptive plasticity between climatic regions based on simulated data, without considering interaction effects. Furthermore, we detected that plasticity effects were condition-dependent between generations, with Sonora exhibiting the most pronounced plasticity when both parents and offspring were acclimated (*matched* cues). When only one generation was acclimated (*mismatched* cues), the population from Catalina showed either similar or greater effects than Sonora. These results are consistent with theoretical considerations for adaptive significance of *TGP* (Uller et al. 2013). When parental acclimation is adaptive, it is expected to increase tolerance of the next generation while minimizing costs associated with physiological or molecular mechanisms of tolerance (e.g. heat-shock response (Krebs and Loeschcke 1994; Dahlhoff and Rank 2007)). These carry-over effects would generate trade-offs with detriment to offspring fitness when their environment does not resemble the parental experience (Uller et al. 2013; Sheriff et al. 2018), suggesting that mechanisms of plasticity in response to environmental stress are preferentially triggered under *matching* cues compared to *mismatched* cues, i.e. "adaptive matching" following Uller et al. (2013).

Given that the *match*/*mismatch* framework has been recently challenged by Engqvist and Reinhold (Engqvist and Reinhold 2016), here we have provided an alternative approach to infer the adaptability of *TGP*, by using long-term evolutionary simulations of *WGP* and *TGP* under realistic scenarios extracted from historical climate data. We found that predictability and amplitude of temperature fluctuations are larger in Sonora than in Catalina (Figures S2, S3, S4 and S5), suggesting stronger selection on both *WGP* and *TGP* in the Sonoran Desert relative to Mediterranean climate in Catalina.

Expectations for empirically detected interactions between populations and plasticity of thermal tolerance are not possible to simulate directly, since available models don't consider direct interactions between plastic responses. However, since the simulations specifically involve adaptive evolution of *WGP* and *TGP*, these are strictly adaptive changes rather than carry-over

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effects (Kuijper and Hoyle 2015). Simulated data are then more likely to be associated with thermal plasticity responses in *matched* acclimation treatments. When *TGP* was detected in larval traits, *matched* acclimation treatments between parents and offspring increased thermal performance in both populations in a higher proportion than that in *mismatched* treatments, which suggests that both populations exhibit adaptive components of plastic responses. However, the Sonoran region expressed the highest plasticity under *matched* acclimation treatments, while exhibiting the lowest response under *mismatched* treatments between generations. This result strongly suggests that *TGP* of tolerance to upper thermal limits exhibit a more predictive component in the Sonoran population, while Catalina seems to express higher unpredictive positive carry-over effects.

Limitations

A common bias in *TGP* estimations involving stress responses is the potential effect that suboptimal or stressful conditions can impose on experimental groups, particularly for early developmental stages (Kaufmann et al. 2014; Heckwolf et al. 2018). The vulnerability of early stages is not always visible and might impose selection pressure for more tolerant genotypes, resulting in a biased estimation of plasticity (Santos et al. 2019). Our approach accounted for such potential bias by acclimating the parental generation as adults. *Drosophila mojavensis* adults have been previously shown to survive temporary exposures to 36°C, both in the lab (Schnebel and Grossfield 1984, 1986; Patton et al. 2001; Krebs and Thompson 2005) and during summertime (Gibbs et al. 2003). Our estimations of *TGP* therefore did not involve differential mortality between experimental conditions and are therefore unbiased. The same rationale applies for our estimations of *WGP* in adults, but potentially not for larval tolerance. Although we controlled for selection on larval tolerance by choosing a suboptimal temperature that *D. mojavensis* larvae tolerated, it was only partially accounted for in eggs. Larval acclimation involved the latter part of egg-to-larva development, and this transition may have been potentially affected by thermal selection. This effect has recently been demonstrated for *ADH* activity (Santos et al. 2019). Our estimations of *WGP* for larval tolerance should be taken with caution since potentially its measurement could have been biased. This means that estimations of *WGP* for larval tolerance may be overestimated in Catalina since this population is presumably more sensitive to thermal conditions compared to Sonora.

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Conclusions

To date, the only established framework to infer the adaptive significance of phenotypic plasticity across generations is based on *match*/*mismatch* experiments (Uller et al. 2013). Such an approach has been recently argued (Engqvist and Reinhold 2016) as being insufficient to disentangle adaptive and predictive transgenerational effects from mere carry-over effects or *silver spoons* in certain conditions. Here we propose a more efficient framework by combining the *match*/*mismatch* approach with more recently available models to perform long-term evolutionary simulations of *WGP* and *TGP* (Kuijper and Hoyle 2015). As previously suggested, environmental predictability is essential to adaptive *TGP*, and we proposed to account for ecological meaningful environmental variability to perform a more realistic set of simulations that can efficiently help to disentangle such effects. Our proposed framework proved to be highly effective to disentangle strictly adaptive and predictive plasticity across generations as the more likely evolved effect explaining tolerance to upper thermal limits in *D. mojavensis* across life stages in an ecological context with substantial regional climate variability. The proposed framework opens the door not only to study ecological scenarios, but also to extend its application to other avenues of research such as experimental evolution studies to detect qualitatively different levels of both *WGP* and *TGP*.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1 [Model description for individual-based simulations for evolution of maternal effects**]**

Appendix S2 [Adaptation to temperature timeseries**]**

Table S1 [*GLM* analysis for viability and development time pupa-adult following heat shocks in *D. mojavensis***]**

Table S2 [Complete *GLM* analysis of variance for heat knockdown in in *D. mojavensis* adults, including acclimation at larva and adult stages (phenotypic plasticity) and parental treatments (transgenerational effects).

Table S3 [Parameter values used for individual-based simulations of *WGP* and *TGP***]**

Figure S1 [Heat-shock tolerance of *D. mojavensis* following 1.5h heat-shocks in larvae and adult males**]**

Figure S2 [Loess decomposed time series of the average temperature in Sonora**]**

Figure S3 [Loess decomposed time series of the average temperature in Catalina**]**

Figure S4 [Autocorrelations of the seasonal temperature component versus time lag in days for Sonora and Catalina**]**

Figure S5 [Histograms comparing the range of standardized temperatures across Sonora than in Catalina**]**

Figure S6 [Evolving phenotypes over time for a single example individual-based simulation in Sonora**]**

Figure S7 [Evolved values of transgenerational and within-generational plasticity after 50,000 generations when varying the overall strength of selection ω^2_z from strong to weak**]**

Figure S8 [Evolved values of transgenerational and within-generational plasticity after 50,000 generations when varying the costs of within-generational plasticity ω^2_b from strong to weak**]**

Figure S9 [Reaction norms affecting larval traits, based on the evolved values of *a*, *blv*, *bad*, *mlv*, *mad* from the individual-based simulations, while varying the strength of selection ω^2_z**]**

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Figure S10 [Reaction norms affecting larval traits, based on the evolved values of *a, blv, bad, mlv, mad* from the individual-based simulations, while varying the cost of *WGP* ω^2_b**]**

TABLES

Table 1. *GLM* analysis for thermal responses (components of viability, standardized viability and development time) following heat shocks after F_1 larval acclimation (*WGP*) and parental treatments (*TGP*) in *D. mojavensis* populations. Degrees of freedom and *P-values* are shown for each trait.

Effect	*Df*	*Viability*			*Std viability*			*Development time*		
		Df_{RES}	*LP*	*LA*	Df_{RES}	*LP*	*LA*	Df_{RES}	*LP*	*LA*
Population (*Pop*)	1	168	***<0.001***	***<0.001***	168	***<0.001***	***<0.001***	122	***<0.001***	***<0.001***
Heat-shock period (*HS*)	1	167	***<0.001***	***<0.001***	167	***<0.001***	***<0.001***	121	0.207	0.258
Acclimation parents ($Accl_{parents}$)	1	166	***<0.001***	***<0.001***	166	***<0.001***	***<0.001***	120	0.556	0.969
Acclimation larva ($Accl_{larva}$)	1	165	***<0.001***	***<0.001***	165	***<0.001***	***<0.001***	119	***<0.001***	***<0.001***
$Pop * HS$	1	164	0.824	0.983	164	0.351	0.319	118	0.662	0.369
$Pop * Accl_{parents}$	1	163	***0.046***	0.071	163	0.547	0.662	117	***0.002***	***<0.001***
$Pop * Accl_{larva}$	1	162	***0.001***	***0.001***	162	***0.044***	0.062	116	0.101	0.068
$HS * Accl_{parents}$	1	161	0.080	0.075	161	0.623	0.631	115	0.677	0.603
$HS * Accl_{larva}$	1	160	***0.000***	***0.000***	160	0.111	0.168	114	0.198	0.360
$Accl_{parents} * Accl_{larva}$	1	159	***0.002***	***0.003***	159	0.714	0.667	113	0.986	0.734
$Pop * HS * Accl_{parents}$	1	158	0.206	0.323	158	0.478	0.595	112	0.236	0.458
$Pop * HS * Accl_{larva}$	1	157	0.328	0.289	157	0.695	0.860	111	0.988	0.837
$Pop * Accl_{parents} * Accl_{larva}$	1	156	0.451	0.337	156	0.052	0.088	110	***<0.001***	***<0.001***
$HS * Accl_{parents} * Accl_{larva}$	1	155	0.142	0.109	155	0.878	0.984	109	0.814	0.453

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$Pop * HS * Accl_{parents} * Accl_{larva}$	1	154	0.262	0.336	154	0.195	0.245	108	0.401	0.350

Significant values ($p < 0.05$) are highlighted in bold

LP: larva-pupa

LA: larva-adult

Table 2. *GLM* analysis for heat knockdown after F_1 acclimation (larvae and adults) (*WGP*) and parental treatments (*TGP*) in *D. mojavensis* populations. Acclimation was tested at larva and adult stages.

Effect	*Df*	Df_{RES}	*P*
Population (*Pop*)	1	430	***0.021***
Acclimation parents ($Accl_{parents}$)	1	428	0.112
Acclimation adults ($Accl_{adults}$)	1	429	***<0.001***
Acclimation larva ($Accl_{larva}$)	1	427	0.914
Sex	1	426	***<0.001***

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$Pop * Accl_{adults}$	1	425	***0.018***
$Pop * Accl_{parents}$	1	424	0.710
$Pop * Sex$	1	422	0.744
$Accl_{parents} * Accl_{adults}$	1	421	0.968
$Accl_{adults} * Sex$	1	419	***0.035***
$Accl_{parents} * Sex$	1	417	0.545
$Pop * Accl_{parents} * Accl_{adults}$	1	415	0.717
$Pop * Accl_{adults} * Sex$	1	413	0.327
$Pop * Accl_{parents} * Sex$	1	411	0.968
$Accl_{parents} * Accl_{adults} * Sex$	1	408	0.567
$Pop * Accl_{parents} * Accl_{adults} * Sex$	1	404	0.798

Significant values ($p < 0.05$) are highlighted in bold

Interactions involving $Accl_{larva}$ were not significant and were not included for simplification (Table 2S).

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FIGURE CAPTIONS

Figure 1. *D. mojavensis* distribution across climatic regions with substantial differences in temperature variability (Desert vs Mediterranean climates). a) Map showing *D. mojavensis* distribution in Santa Catalina Island and Sonoran Desert. b) Daily and seasonal variation of temperature experienced by sampled regions in Catalina and Sonora during 2010 (Data provided by National Centers for Environmental Information, NOAA from their web site https://www.ncdc.noaa.gov/cdo-web/datasets#NORMAL_HLY). c) Factorial design used to investigate the effect of acclimation as performed at either 25 or 36°C for 24h in parents and F_1 offspring on tolerance to upper thermal limits.

Figure 2. Heat-shock tolerance of *D. mojavensis* populations (Catalina vs Sonora) following acclimation treatments performed in parents and F_1 offspring. Heat shocks were performed using a ramping treatment (30°C to 40°C at 0.13°C/min) followed by 2h at 40°C for experiments in larvae or until reaching knockdown for experiments in adult females. a) Results obtained for viability larva-adult (standardized), development time larva-adult and heat knockdown (± *SE*). b) Results of variation partitioning analysis showing the proportion of variation explained by within- (*WGP*) and transgenerational plasticity (*TGP*) for each trait. Only results for 2h heat-shocks in larvae and adult females are shown. Results for 1.5h heat-shocks and adult males are shown in Figure S1.

Figure 3. Individual-based simulations showing evolved values of reaction norm slopes (± *SD*) and maternal effects expressed in a) larvae and b) adults. The model predicts that populations from Sonora have evolved both stronger WGP and TGP (at least in larval traits) relative to populations in Catalina, mimicking the empirical findings (Figure 2a). Evolved reaction norms (15 replicate simulations) are then used to simulate the temperature exposure experiment

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(Appendix S2). Parameters: $\omega_z^2=\omega_b^2=\omega_m^2=10$, $\sigma_e^2=0.1$, $s_{min}=0.5$. The remaining used parameters are in Table S3.

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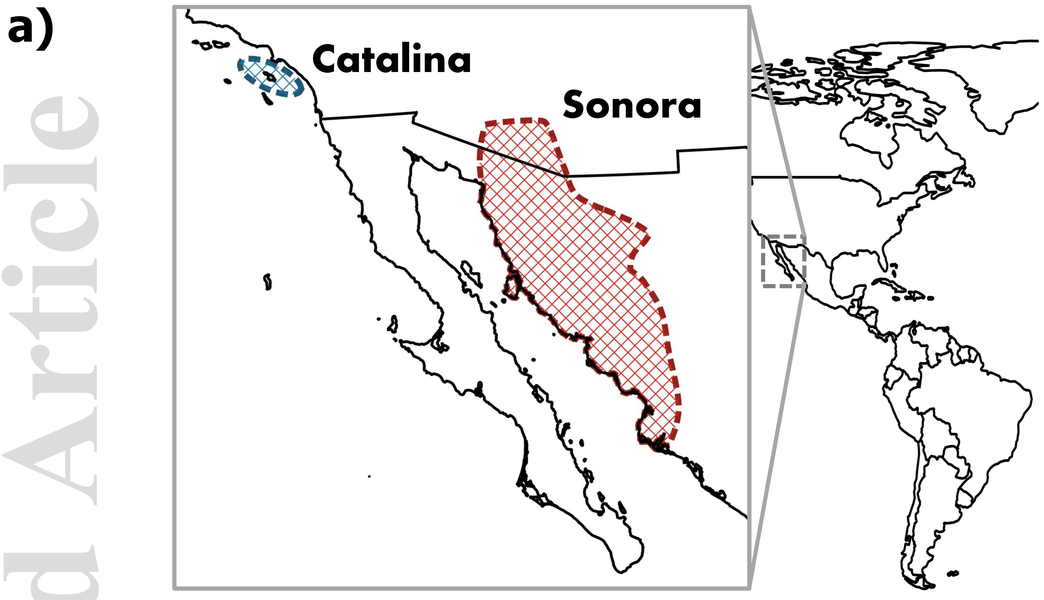

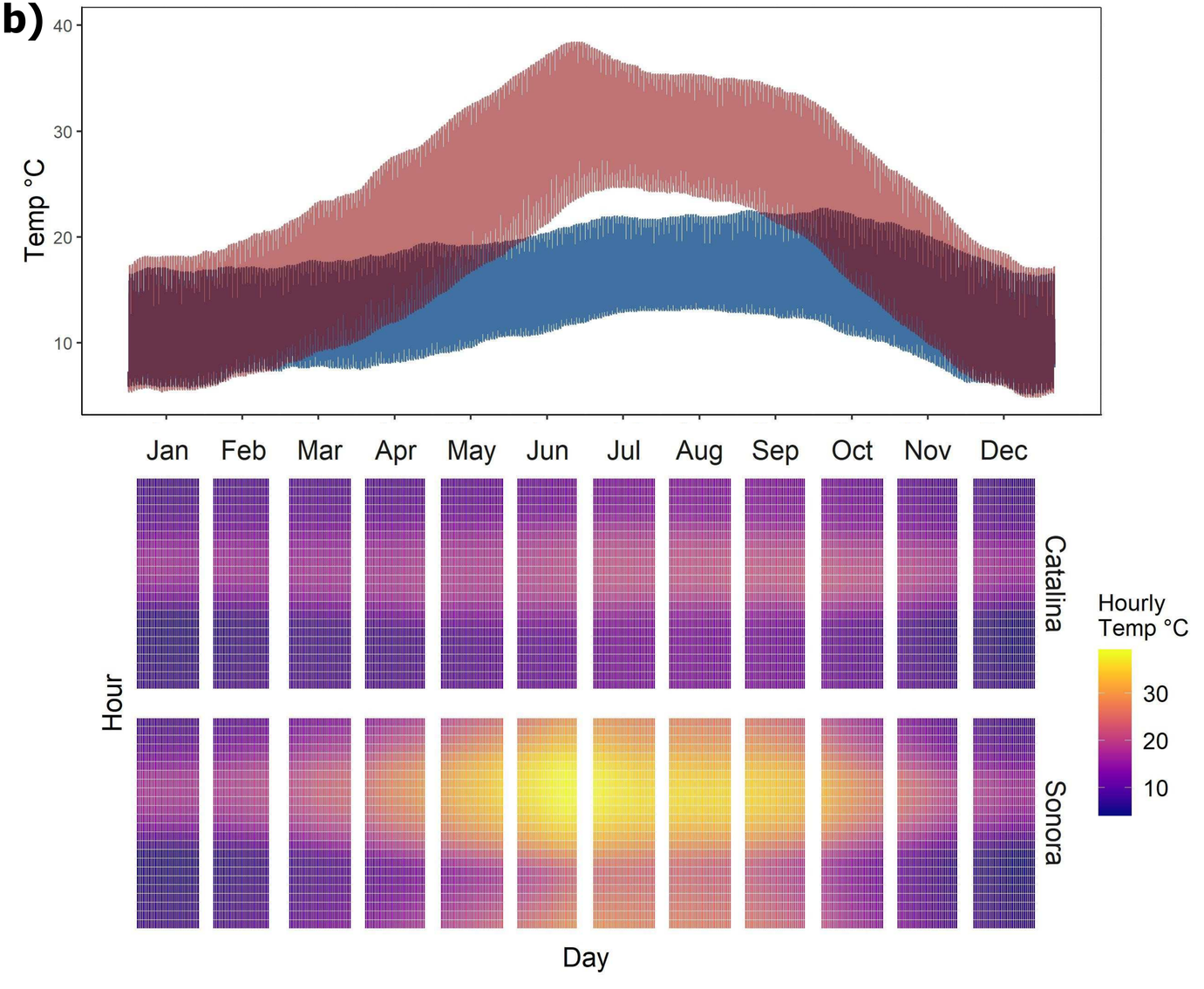

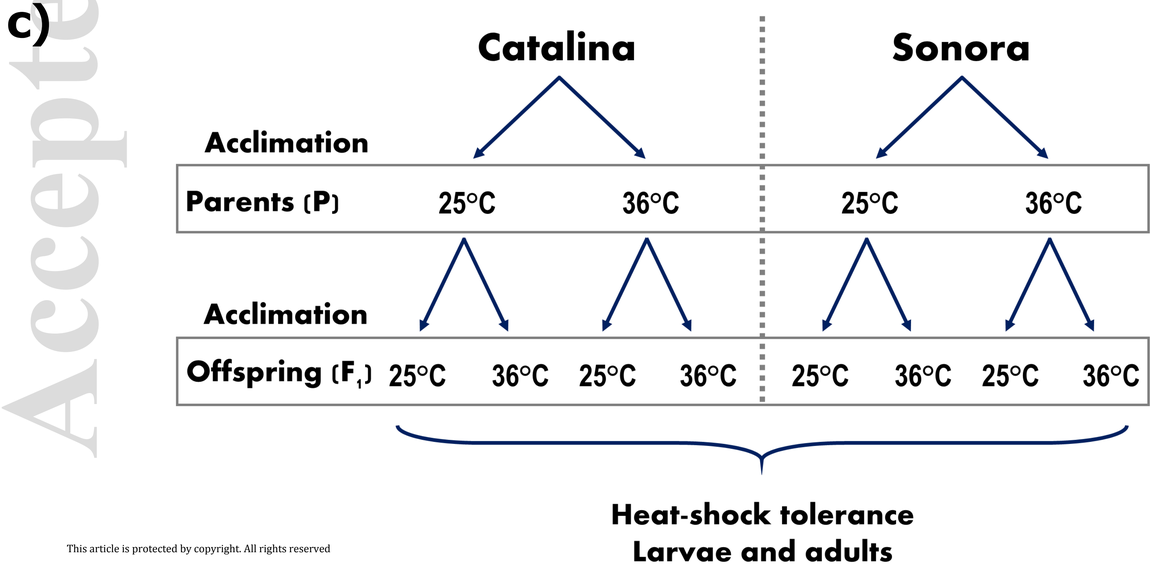

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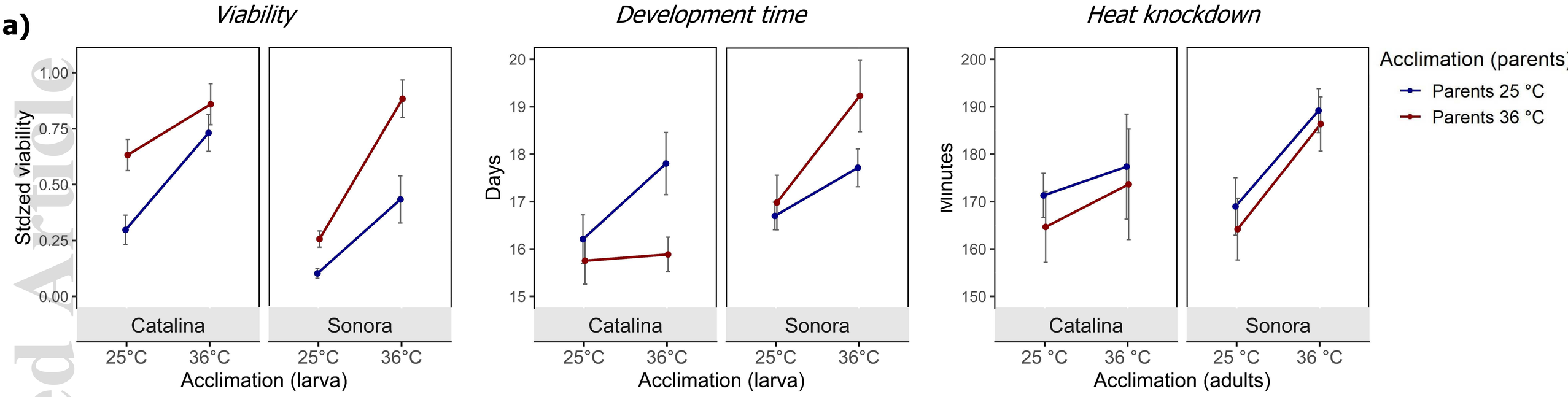
a)



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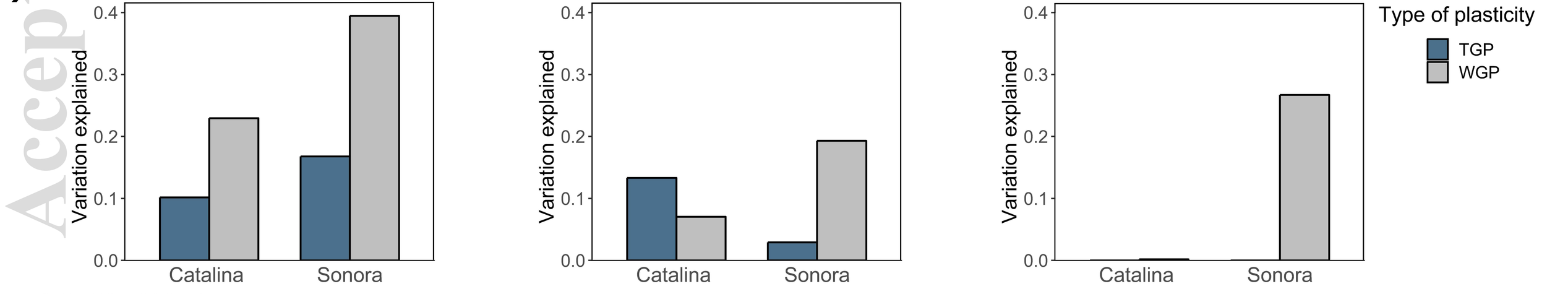

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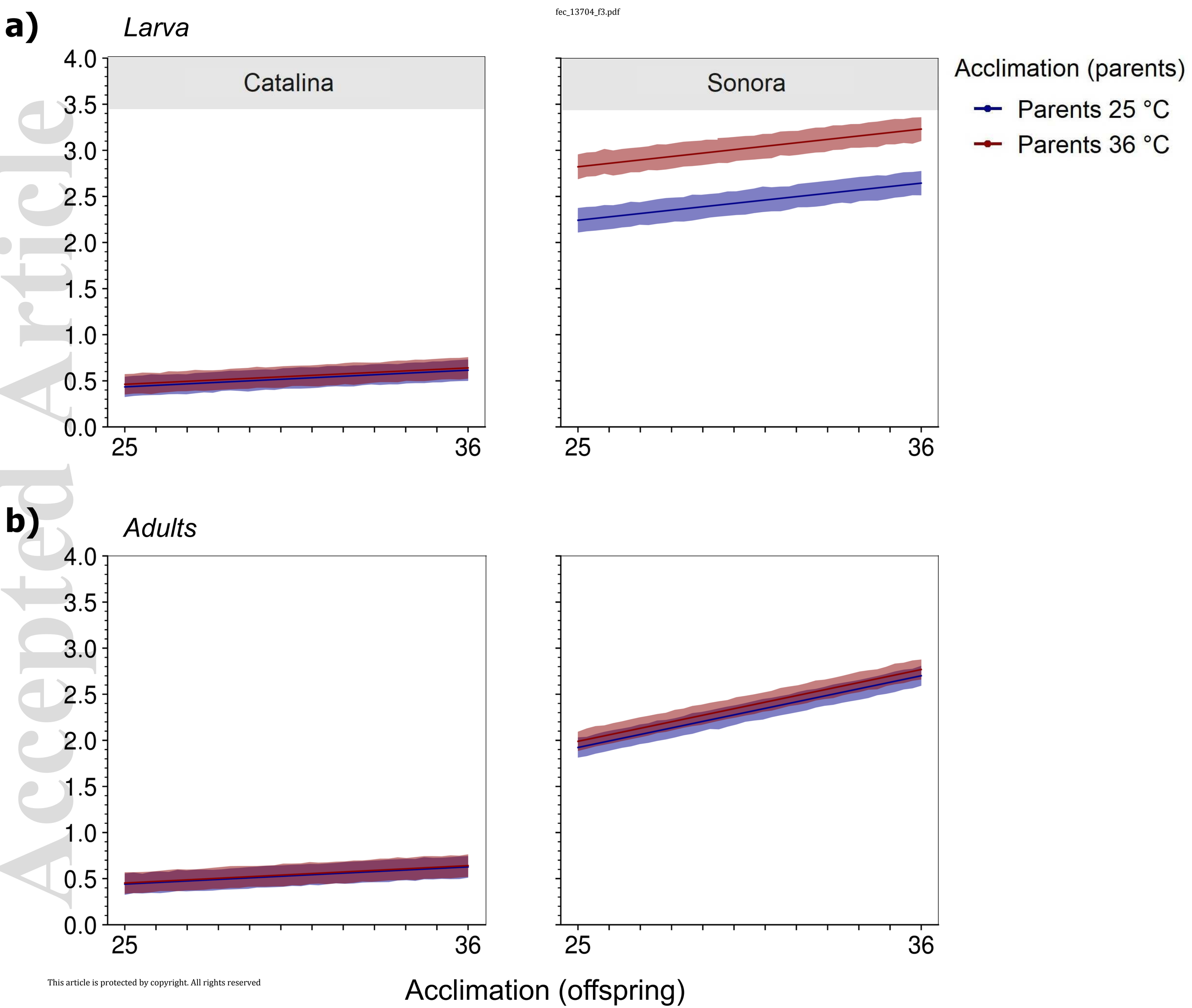

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