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

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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. 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 (vs. at 25°C). We then used historical temperature variation data from both regions to perform individual-based simulations by modelling expected components of adaptive plasticity in multiple life stages.

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.

KEYWORDS
acclimation, carry-over effects, *Drosophila mojavensis*, heat-shock tolerance, individual-based simulations, within/transgenerational plasticity
INTRODUCTION

The role of the environment in shaping phenotypic variation has been recognized since the very beginning of the genotype versus 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 & 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 & Lande, 1989; Moore et al., 2019; Nelson & Nadeau, 2010). These sources of transgenerational variation were traditionally treated as troublesome, unwanted effects masking the genetic variation, so much so that experiments were designed 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 & 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 (Arsenault et al., 2018), mRNA (Ahi et al., 2018), transposons (Migicovsky et al., 2014) or small RNAs (Stief et al., 2014).

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

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 (Heard & Martienssen, 2014; Nelson & Nadeau, 2010; 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 (Donelson et al., 2018; Heard & Martienssen, 2014; Nelson & Nadeau, 2010). 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 (Moore et al., 2019; Mousseau & Fox, 1998; Newcombe et al., 2015; Proulx & Teotónio, 2017), and allows for predictions as to how the parental environment can influence offspring performance (Donelson et al., 2018).

Given the potential of TGP to contribute to the rapid adaptation of populations to a changing global climate (Bonamour et al., 2019; Donelson et al., 2018; Hoffmann & Sgrò, 2011; Sgrò et al., 2016), TGP is considered as a potential source of ecologically and evolutionary meaningful variation (Bonduriansky et al., 2012; Burgess & Marshall, 2011; Herman & Sultan, 2011). 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 & Sgrò, 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 & Sultan, 2011; Münzbergová & 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 & 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 & Hadincová, 2017). However, theoretical considerations (Badyaev & Uller, 2009; Sheriff et al., 2018) supported by theoretical models (Kuijper & Hoyle, 2015; Proulx & 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 & Oh, 2008; Burgess & Marshall, 2011), historical environmental variation is often ignored when defining ecologically relevant cues to trigger TGP in the laboratory (Donelson et al., 2018). Regular and predictable environmental fluctuations such as seasonality offer a potential scenario that facilitates parental–offspring environment predictability (Marshall & 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 (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 & 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 (Sheriff et al., 2018; Uller et al., 2013) 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 & 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 unpredictable carry-over effects under mismatched acclimation treatments (Engqvist & Reinhold, 2016; Sheriff et al., 2018; Uller et al., 2013). We adapted the simulation model to the particular ecological conditions of D. mojavensis using historical climate data from the sampled regions 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.

2 | MATERIALS AND METHODS

2.1 | 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 Mediterranean climate in Catalina Island (Figure 1b). The established mass-bred populations were reared at 25°C, under 12:12 hr 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 & 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.

2.2 | 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 and 36°C, respectively. The experiment had a factorial design with two parental treatments (25 and 36°C in 10- to 12-day-old adults) and two offspring treatments (25 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 hr prior to oviposition. Following this 24-hr acclimation period, a new food plate was placed in each cage for flies to oviposit at 25°C for another 24 hr and these plates were then divided into two equal parts. Each half-plate containing F1 eggs was placed at either 25 or 36°C for 36 hr. The prolonged acclimation period for larvae with respect to that in adults was used to account for the different thermal limits between life stages. Larvae are much more resistant to heat shocks (see Section 3) 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 F1 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.

To test for possible interactions between parental, F1 larval and F1 adult heat acclimation, the above eclosed adults from the eight parental/F1 larval combinations were split one more time. When the F1 adults were approximately 10 days of age, half of them were subjected to either 36 or 25°C treatments for 24 hr. The next day, males and females from the 16 treatments were tested for heat-shock tolerance.

2.3 | 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 (L. M. Matzkin, unpubl. 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 and 2 hr at 40°C. Post-ramping periods were selected based on preliminary data 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 were 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, F1 larvae and adults (16 combinations).

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

\[
y = \mu + (\text{Pop} + \text{Accl}_{\text{parents}} + \text{Accl}_{\text{larva}} + \text{HS})^4.
\]

where \( y \) is the thermal tolerance (viability or development time components larva–pupa–adult), \( \mu \) is the mean thermal tolerance, \( \text{Pop} \) is the population effect (Sonora vs. Catalina), \( \text{Accl}_{\text{parents}} \) is the acclimation effect performed in parental generation and therefore represents TGP, while \( \text{Accl}_{\text{larva}} \) is the WGP effect of acclimation of F1 larva, and HS is the post-ramping heat-shock period performed in larva (1.5 or 2 hr).

For adult traits, the model included the three instances of acclimation (parents, F1 larva and adults):

\[
y = \mu + (\text{Pop} + \text{Sex} + \text{Accl}_{\text{parents}} + \text{Accl}_{\text{larva}} + \text{Accl}_{\text{adults}})^5.
\]

where \( y, \mu, \text{Pop}, \text{Accl}_{\text{parents}}, \text{Accl}_{\text{larva}} \) and \( \text{Accl}_{\text{adults}} \) are the same terms used for larval tolerance, while \( \text{Accl}_{\text{adults}} \) represents the effect of acclimation performed in F1 adults.

Viability components larva–pupa–adult were analysed 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 analysed 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 \texttt{package multcomp}.
2.5 | 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).

2.6 | 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 & Lande, 1989; Kuijper & 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 [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 $z_{t,v}$ while the adult phenotype is $z_{ad}$. Specifically, the larval phenotype $z_{t,v}$ in generation $t$ at the time of birth $t_{b}$ (where $t = \frac{1}{7}$ is the number of days relative to the total lifespan $t$ measured in days) is given by

$$z_{t,v} = a_{t,v} + b_{t,v} t_{b} + m_{t,v} z_{t-1} + e_{t,v}.$$  

(1)

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

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 $s_{t,v}(z, b, m)$ at generation $t$ and day $t$, is given by

$$s_{t,v}(z, b, m) = s_{min} + (1-s_{min}) \exp \left\{ -\frac{1}{2} \left[ \frac{z - \mu_{t,v}}{\sigma^2_b} \right]^2 + \frac{b^2}{\sigma^2_b} + \frac{m^2}{\sigma^2_m} \right\}. \quad (2)$$

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 maximize survival probability) is $z_{t,v}^*$, the temperature of that day (see Appendix S2 ‘Adaptation to temperature time series’), while $\sigma^2_b$ is the width of the selection function, small (large) values of which imply strong (weak) selection. Next, the terms $\frac{b^2}{\sigma^2_b}$ and $\frac{m^2}{\sigma^2_m}$ reflect stabilizing selection against within-generational plasticity and maternal effects, respectively (Kuijper & Hoyle, 2015).

Larvae which have survived according to Equation (2) for $t_{ad}<c$ days become adults, after which they develop an adult phenotype $z_{ad+t,v}$ 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:

$$z_{ad+t,v} = a_{t,v}^a + b_{t,v}^a t_{b} + m_{t,v}^a z_{t-1}^a + e_{t,v}^a.$$  

(3)

where $a_{t,v}^a$, reflects the elevation, which is the same trait as expressed in larvae, conditional on that the individual has survived for $t_{ad}<c$ days (denoted by '). The strength of within-generational plasticity in adulthood is $b_{t,v}^a$, which reflects the strength of the reaction norm in response to the environment $t_{b}$ at the onset of adulthood. Regarding transgenerational plasticity, $m_{t,v}^a$ reflects sensitivity to the maternal phenotype at adulthood. Here, the maternal phenotype $z_{t-1}$ 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, $e_{t,v}$ again reflects developmental noise.

The traits $b_{t,v}$, $b_{t,v}^a$, $m_{t,v}$, and $m_{t,v}^a$ 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 & Ezard, 2012; Lande, 2009). For the sake of simplicity, all loci are unlinked with 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 & Ezard, 2012; Lande, 2009). For the sake of simplicity, all loci are unlinked.

3 | RESULTS

Acclimation treatments performed at 36°C (vs. 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
TABLE 1 GLM analysis for thermal responses (components of viability, standardized viability and development time) following heat shocks after F1 larval acclimation (WGP) and parental treatments (TGP) in Drosophila mojavensis populations. Degrees of freedom and p values are shown for each trait.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>df_RES</th>
<th>Pop</th>
<th>La</th>
<th>Pop</th>
<th>La</th>
<th>Pop</th>
<th>La</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability</td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Population (Pop)</td>
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<td>168</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>168</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>122</td>
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<tr>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>121</td>
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<td>Acclimation parents (Acclparents)</td>
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<td>&lt;0.001</td>
<td>120</td>
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<tr>
<td>Acclimation larva (Accllarva)</td>
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<td>&lt;0.001</td>
<td>165</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>164</td>
<td>0.351</td>
<td>0.319</td>
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<td>Pop × Accllarva</td>
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<td>162</td>
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<tr>
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<td>0.000</td>
<td>0.000</td>
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<td>0.111</td>
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<tr>
<td>Acclparents × Accllarva</td>
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<td>0.195</td>
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Note: Significant values (p < 0.05) are highlighted in bold.
Abbreviations: LA, larva-adult; LP, larva–pupa.

FIGURE 2 Heat-shock tolerance of Drosophila mojavensis populations (Catalina vs. Sonora) following acclimation treatments performed in parents and F1 offspring. Heat shocks were performed using a ramping treatment (30°C to 40°C at 0.13°C/min) followed by 2 hr 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 2 hr heat shocks in larvae and adult females are shown. Results for 1.5 hr heat shocks and adult males are shown in Figure S1.
TABLE 2 GLM analysis for heat knockdown after F$_1$ acclimation (larvae and adults; WGP) and parental treatments (TGP) in Drosophila mojavensis populations. Acclimation was tested at larva and adult stages

<table>
<thead>
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<th>df$_{RES}$</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td>0.021</td>
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<tr>
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<td>Acclimation adults (Accl$_{adults}$)</td>
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<td>&lt;0.001</td>
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<td>Acclimation larva (Accl$_{larva}$)</td>
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</tr>
<tr>
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<td>425</td>
<td>0.018</td>
</tr>
<tr>
<td>Pop × Accl$_{parents}$</td>
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<tr>
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<tr>
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</table>

Note: Significant values (p < 0.05) are highlighted in bold. Interactions involving Accl$_{larva}$ were not significant and were not included for simplification (Table 52).

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

3.1 Larval tolerance to upper thermal limits

Viability was analysed 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 2 hr heat shock) but tended to increase population and acclimation effects. Hereafter, we focus on results obtained in for 2 hr 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 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 2 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 2 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).

3.2 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 52), 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).

3.3 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 also 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.

3.4 | 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 F1 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 & 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 analysed (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; Figures S7 and 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.

4 | 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 (Figure 1b; Figure 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.

4.1 | 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 (Dahlhoff & Rank, 2007; Krebs & Loeschcke, 1994), 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; Kellermann & Sgrò, 2018; Sgrò et al., 2010), even in D. mojavensis (Krebs, 1999; Krebs & 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 (Bahndorf et al., 2010; Cai et al., 2017; Dahlgaard et al., 1998; Díaz et al., 2015). These components are known to accumulate rapidly during mid-range temperatures (e.g. 36°C) as occurs in D. mojavensis (Krebs, 1999; Krebs & 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 & Loeschcke, 2002; Zizzari & 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 (Feder et al., 1997; Krebs & Loeschcke, 1995). Moreover, the molecular machinery of heat-shock response is known to involve considerable energy cost (Dahlhoff & Rank, 2007; Krebs & Loeschcke, 1994), which often leads to trade-offs between life stages and reproductive-related behaviours (Jørgensen et al., 2006; Zhang et al., 2015) leading to more limited WGP in adults (Sørensen & Loeschcke, 2002) as has been previously found in D. mojavensis (Fasolo & Krebs, 2004; Patton et al., 2001).

4.2 | 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 versus 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 (Bonduriansky et al., 2012; Galloway & Etterson, 2007; Mousseau & Fox, 1998; Nestler, 2016), more particularly for climate change scenarios (Bonamour et al., 2019; Burgess & Marshall, 2011; Münzbergová & Hadincová, 2017), 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 & Reinhold, 2016; Sheriff et al., 2018). A more formal link to the adaptive significance of these effects should 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 5–10). 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 & 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 & Wieschaus, 1986; Tadros & Lipshitz, 2009; Wieschaus, 1996), 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 oocytes 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.

4.3 Adapting 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 higher plastic responses (Figure 1b; Figure S5). Except for adult data (TGP not detected for heat knockdown), all traits analysed 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 (Ullner 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 Dahlhoff & Rank, 2007; Krebs & Loschke, 1994)). These carry-over effects would generate trade-offs with detriment to offspring fitness when their environment does not resemble the parental experience (Sheriff et al., 2018; Ullner et al., 2013), suggesting that mechanisms of plasticity in response to environmental stress are preferentially triggered under matching cues compared to mismatched cues, that is, ‘adaptive matching’ following Ullner et al. (2013).

Given that the match/mismatch framework has been recently challenged by Engqvist and Reinhold (Engqvist & Reinhold, 2016), here we have provided an alternative approach to infer the adaptability of TGP, 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–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 do not 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 effects (Kuijper & 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 predictable component in the Sonoran population, while Catalina seems to express higher unpredictable positive carry-over effects.

4.4 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 (Heckwolf et al., 2018; Kaufmann et al., 2014). 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 laboratory (Krebs & Thompson, 2005; Patton et al., 2001; Schnebel & Grossfield, 1984, 1986) 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.
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 & 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 & 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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AUTHORS’ CONTRIBUTIONS

F.D. and L.M.M. conceived the idea and designed laboratory experiments; F.D., N.T. and J.M.C. performed all the laboratory experiments; F.D. conducted all the statistical analyses of phenotypic data; B.K. and R.B.H. designed all the simulation modelling and computational work; B.K. conducted the computational work and analysed the simulated data; F.D., N.T., J.M.C., B.K., R.B.H. and L.M.M. were all involved in the analysis and writing of the manuscript.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.stqj2c22 (Diaz et al. 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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