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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2 **tolerance across life stages and climatic regions**

3

4 **Abstract**

5

6 Although environmental variability and predictability have been proposed as the underlying ecological

7 context in which transgenerational plasticity (*TGP*) arises, the adaptive significance and interaction with

8 within-generation plasticity (*WGP*) in such scenarios is still poorly understood. In order to investigate

9 these questions, we considered the tolerance to upper thermal limits of larvae and adults of the desert

10 endemic *Drosophila mojavensis* adapted to different climatic regions (Desert vs Mediterranean climate).

11 Thermal plasticity was investigated by acclimating parents and offspring at 36°C (versus at 25°C). We

12 then used historical temperature variation data from both regions to perform individual-based simulations

13 by modeling expected components of adaptive plasticity in multiple life stages. Thermal response to

14 ramping heat shocks was more pronounced in larvae, where acclimation treatments in parents and

15 offspring increased their heat-shock performance, while heat knockdown in adults was only increased by

16 offspring acclimation of adults. The relative contribution of *WGP* and *TGP* was greater for the population

17 from the more thermally variable Sonoran Desert. Similarly, individual-based simulations of evolving

18 maternal effects indicated that variation in tolerance to upper thermal limits across life stages and

19 climates is expected from its adaptive significance in response to environmental predictability. Our

20 approach offers a new perspective and interpretation of adaptive plasticity, demonstrating that

21 environmental predictability can drive thermal responses across generations and life stages in a scenario

22 with regional climate variability.

23 **Key words:** Within/transgenerational plasticity, acclimation, carry-over effects, heat-shock tolerance,

24 individual-based simulations, *Drosophila mojavensis*.

2

25   **Introduction**

26

27   The role of the environment in shaping phenotypic variation has been recognized since the very

28   beginning of the genotype vs environment discussion (Baldwin 1896). The importance of these dynamics

29   has led to the view that an organism's phenotype is the result of a unique interaction between its genotype

30   and its whole temporal trajectory of external environments (Fusco and Minelli 2010). Although genetic

31   variation was initially considered the ultimate source of change, non-genetic inherited changes such as

32   maternal effects have been well recognized as a source of phenotype variation for decades (Kirkpatrick

33   and Lande 1989; Nelson and Nadeau 2010; Moore et al. 2019). These sources of transgenerational

34   variation were traditionally treated as troublesome, unwanted effects masking the genetic variation, so

35   much so that experiments were designed in order to remove them (Falconer 1981). The reconsideration

36   of these effects has illustrated how the parental environment can contribute to the phenotype of the next

37   generation, acting as a transgenerational form of phenotypic plasticity (Heard and Martienssen 2014).

38   Currently, it is well recognized that parents can alter the phenotype of their offspring through a number

39   of non-genetic or epigenetic processes (Nestler 2016), such as DNA methylation (Arsenault et al. 2018),

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

41

42   There is increasing evidence demonstrating the role played by the carry-over effects of environmental

43   exposure across different time scales over a single generation (Nelson and Nadeau 2010). The genetic

44   basis of within-generation plasticity (*WGP*) and its role in buffering or favoring natural selection via

45   genetic assimilation has been extensively explored (Pigliucci et al. 2006; Badyaev 2009). Ecological

46   conditions in which natural selection can influence the level of an organism's response to environmental

47   fluctuations leading to adaptive *WGP* have been reported in many taxa (Via 1993; Delpuech et al. 1995;

48   Moreteau et al. 2003; Crispo 2008; Lind et al. 2011). This evidence has established a solid theory

49   including both empirical and substantial theoretical modelling (Jong 1995; Lande 2009; Chevin et al.

50   2010; Herron and Doebeli 2011), defining the interaction between selection and *WGP* (Schlichting and

51   Pigliucci 1998; Pigliucci et al. 2006; Fusco and Minelli 2010).

52

53   On the other hand, the role transgenerational plasticity (*TGP*) in evolution is less understood. Most of

54   the effort has been focused on demonstrating transmissible effects over generations, which has been

55   corroborated for many traits (Yin et al. 2019), as well as its associated molecular mechanisms (Nelson

56   and Nadeau 2010; Heard and Martienssen 2014; Nestler 2016). These transgenerational effects are

57   currently lacking a unified definition, being currently referred to through numerous different terms such

58   as non-genetic inheritance, maternal effects, anticipatory parental effects, carry-over effects,

59   intergenerational effects, among others (Nelson and Nadeau 2010; Heard and Martienssen 2014;

60   Donelson et al. 2018). Here we focus on a definition that allows the study of whether such responses are

61   adaptive as opposed to merely carry-over effects: as reviewed by Donelson et al. (2018), we consider

62   *TGP* to describe the effect of interactions between environmental conditions experienced by parental and

63   offspring generations on the offspring phenotype. This definition is in line with that of traditional

64   maternal (or paternal) effects and their role in adaptation (Mousseau and Fox 1998; Newcombe et al.

65   2015; Proulx and Teotónio 2017; Moore et al. 2019), and allows for predictions as to how the parental

66   environment can influence offspring performance (Donelson et al. 2018).

67

68   Given the potential of *TGP* to contribute to the rapid adaptation of populations to a changing global

69   climate (Hoffmann and Sgró 2011; Sgrò et al. 2016; Donelson et al. 2018; Bonamour et al. 2019), *TGP*

70   is considered as a potential source of ecologically and evolutionarily meaningful variation (Burgess and

71   Marshall 2011; Herman and Sultan 2011; Bonduriansky et al. 2012). Predicted climate change has

72   inspired a multitude of studies demonstrating the role of acclimation (Anderson et al. 2012) in enabling

73   organisms to overcome periods of environmental change within a single generation (Hoffmann and Sgró

74   2011; Overgaard et al. 2011). Since such changes can persist across multiple generations, adaptive *TGP*

75   has been proposed as an important mechanism to overcome stress environments in a number of species,

76   including plants (Herman and Sultan 2011; Münzbergová and Hadincová 2017), nematodes (Massamba-

77   N'Siala et al. 2014; Webster et al. 2018), vertebrates (Badyaev 2009; Steenwyk et al. 2018), marine

78   species (Guillaume et al. 2016; Ryu et al. 2018) and insects (Schiffer et al. 2013; Zizzari and Ellers 2014).

79   The role of these plastic responses is commonly assumed to be similar to what has been found for *WGP*,

80   buffering populations against extreme fluctuations in the near term or canalizing natural selection in the

81   long term (Münzbergová and Hadincová 2017). However, theoretical considerations (Badyaev and Uller

82   2009; Sheriff et al. 2018) supported by theoretical models (Kuijper and Hoyle 2015; Proulx and Teotónio

83   2017) have pointed to environmental variability and predictability across generations as the evolutionary

84   scenario that promotes adaptive *TGP* over and above *WGP*.

85

86   With a few exceptions (Badyaev and Oh 2008; Burgess and Marshall 2011), historical environmental

87   variation is often ignored when defining ecologically relevant cues to trigger *TGP* in the lab (Donelson

88   et al. 2018). Regular and predictable environmental fluctuations such as seasonality offer a potential

89   scenario that facilitates parental-offspring environment predictability (Marshall and Burgess 2014), since

90   the level of autocorrelation across the life cycle has been considered a determinant for adaptive *TGP*.

91   Indeed, recent reviews have pointed to *match*/*mismatch* experiments from factorial designs in which both

92   parents and offspring are exposed to alternative environments (often stress and non-stress) as an

93   indication of predictability and therefore adaptive *TGP* (Sheriff et al., 2018; Uller et al., 2013). The

94   impact of predictability resulting from *matched*, when compared to *mismatched* cues, is suggested from

95   the costs of *TGP* when the parental environment does not efficiently predict that in the offspring

96   (*mismatched* cues). However, this approach has been argued as insufficient when disentangling adaptive

97   *TGP* from other non-predictive carry-over effects such as *silver spoons* (where individuals that develop

98   in good conditions experience fitness benefits as adults) in certain conditions (Engqvist and Reinhold

99   2016), which again has left several questions regarding the interplay between *WGP* and *TGP* unresolved:

100   Do they respond to the same kind of fluctuations? Are they convergent responses to fluctuations? What

101   is their relative importance in a given ecological context?

102

103   Here we propose to combine experimental evidence from *match*/*mismatch* experimental framework

104   (Uller et al. 2013; Sheriff et al. 2018) where parents and offspring are both exposed to either moderate

105   or stress temperatures, with individual-based simulations data for the evolution of *WGP* and *TGP*

106   (Kuijper and Hoyle 2015), to investigate the adaptive component of plasticity of heat tolerance in two

107   genetically and ecologically distinct populations of the desert *Drosophila mojavensis* (Heed 1978;

108   Matzkin 2014). The central hypothesis is that evolution under a more fluctuating environment (Sonoran

109   Desert relative to buffered Mediterranean climate of Santa Catalina Island, California) will exhibit higher

110   thermal plasticity under *matching* environments between parents and offspring, while minimizing

111   unpredictive carry-over effects under *mismatched* acclimation treatments (Uller et al. 2013; Engqvist and

112   Reinhold 2016; Sheriff et al. 2018). We adapted the simulation model to the particular ecological

113   conditions of *D. mojavensis* using historical climate data from the sampled regions in order to generate

114   predictions for adaptive responses in larvae and adults. Our results point to adaptive differentiation in

115   thermal plasticity linked to environmental predictability across life stages in an ecological context with

116   substantial regional climate variability.

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121   **Materials and methods**

122

123   *Samples*

124   Each experimental population was established by pooling four isofemale lines of *D. mojavensis*

125   originally collected in Santa Catalina Island, California or Sonoran Desert, Mexico (hereafter, Catalina

126   and Sonora) (Figure 1a). Whereas the population from the Sonoran Desert experiences higher

127   temperatures (mean and maximum) and variance (diurnal and annual) relative to that from Mediterranean

128   climate in Catalina Island (Figure 1b). The established mass-bred populations were reared at 25°C, under

129   12:12 h light:dark cycle and controlled density conditions in 8-dram glass vials with banana-molasses

130   media for four generations before experiments (Coleman et al. 2018). Since *D. mojavensis* females

131   multiply mate (Knowles and Markow 2001), each of the founder isofemale lines per population will tend

132   to be segregating variation from multiple sires. Hence at minimum, each of the populations captured

133   variation from at least 16 independent segregating haploid genomes, but likely more depending on how

134   often the female mate, which we considered enough for interpopulation comparisons. A more expanded

135   sampling will be necessary in future studies for deep intrapopulation genetic analyses and mapping.

136

137   *Experimental design*

138   Heat-shock tolerance was assessed in response to previous acclimation exposure performed in parents

139   and offspring at either moderate or stress temperatures of 25°C and 36 °C respectively. The experiment

140   had a factorial design with two parental treatments (25°C and 36°C in 10-12 days-old adults) and two

141   offspring treatments (25°C and 36°C in larvae and adults) for each population (Figure 1c). The parental

142   generation of both populations was divided into two cages with a banana-molasses food plate and each

143   cage was subjected to either 25 or 36°C treatments in a Percival incubator for 24 h prior to oviposition.

144   Following this 24 h acclimation period, a new food plate was placed in each cage for flies to oviposit at

145   25°C for another 24 h and these plates were then divided into two equal parts. Each half-plate containing

146   F_1 eggs was placed at either 25°C or 36°C for 36 h. The prolonged acclimation period for larvae with

147   respect to that in adults was used in order to account for the different thermal limits between life stages.

148   Larvae are much more resistant to heat shocks (see results) and therefore required prolonged time to

149   trigger heat-shock responses. The chosen temperature and periods correspond to the maximum treatment

150   that trigger a heat-shock response without killing individuals in the process. Hatched first instar larvae

151   were then placed in groups of 30 into food vials. Approximately 40 vials per each of the 8 half-plates

152   representing the different combinations of parental and F_1 larval treatments were collected. Half of these

153   vials were immediately used to test for the heat-shock tolerance of first instar larvae. The second half of

154   these vials were maintained at 25°C until flies eclosed to perform experiments on adults.

155

156   To test for possible interactions between parental, F_1 larval and F_1 adult heat acclimation, the above

157   eclosed adults from the 8 parental/F_1 larval combinations were split one more time. When the F_1 adults

158   were approximately 10 days of age, half of them were subjected to either 36°C or 25°C treatments for 24

159   h. The next day, males and females from the 16 treatments were tested for heat-shock tolerance.

160

161   *Heat-shock experiments*

162   Thermal performance of first instar larvae and adults was assessed using a ramping treatment in a water

163   bath with temperature controlled by a Thermo Scientific Circulator (AC 200). The ramping treatment

164   was set between 30°C up to 40°C. First, temperature was held at 30°C for 15 min and then it was

165   increased by 0.13°C/min until reaching 40°C, where temperature remained constant for the rest of the

166   experiment depending on the fly stage in test (see below). The ramping rate was estimated from field

167   measurements of rotting cacti in Organ Pipe National Monument (Arizona, USA) during summertime

168   (Authors' unpublished data).

169   For larvae, vials with food containing groups of 30 larvae were submerged in the water bath for a post

170   ramping period of 1.5 h and 2 h at 40°C. Post ramping periods were selected based on preliminary data

171   in order to capture mid and high stressful treatments and correspond to the *HS* term in the linear model

172   (see statistical analysis below). For the larval assays, the number and time of pupation and hatched adults

173   was recorded on a daily basis for 10-12 replicates per treatment. For adult performance, males and

174   females were placed in individual 1-dram capped vials, then randomly arranged on clamps on an acrylic

175   frame and submerged in a transparent water bath allowing the visual inspection of the vials. All flies

176   were constantly observed and scored for time until heat knockdown was reached. Knockdown was

177   defined as the moment in which flies were not able to hold themselves upright or move after being

178   stimulated by a strong flashlight. A total of 15 replicates were scored per treatment combination of

179   acclimation performed in parents, F$_1$ larvae and adults (16 combinations).

180

181   *Statistical analysis and modelling*

182   Acclimation effects for larvae and adults were tested using a generalized linear model (*GLM*). These

183   models evaluated *WGP* and *TGP* as a result of acclimation in parents and offspring as well as additional

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

185

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

187

188   where y is the thermal tolerance (viability or development time components larva-pupa-adult), μ is the

189   mean thermal tolerance, *Pop* is the population effect (Sonora vs Catalina), $Accl_{parents}$ is the acclimation

190   effect performed in parental generation and therefore represents *TGP*, while $Accl_{larva}$ is the *WGP* effect

191   of acclimation of F$_1$ larva, and *HS* is the post ramping heat-shock period performed in larva (1.5 or 2 h).

192   For adult traits, the model included the three instances of acclimation (parents, F$_1$ larvae and adults):

193
$$y = \mu + (Pop + Sex + Accl_{parents} + Accl_{larva} + Accl_{adults})^5,$$

194

195 where y, μ, Pop, $Accl_{parents}$ and $Accl_{larva}$ are the same terms used for larval tolerance, while $Accl_{adults}$

196 represents the effect of acclimation performed in F_1 adults.

197

198 Viability components larva-pupa-adult were analyzed directly using a logit *GLM* link function as well as

199 a proportion between heat-shocked larvae with respect to that of viability of non-heat-shocked samples

200 (acclimated samples but not subjected to heat shocks) – hereafter standardized viability. Because

201 standardized viability does not follow a binomial distribution, we used a logarithm transformation in

202 order to fit normal distribution of data followed by a gaussian *GLM* function. Components of

203 development time (larva-pupa-adult) as measured from heat-shocked larvae and heat knockdown in

204 adults were analyzed through a gaussian *GLM* link function on untransformed data since data were

205 mostly normally distributed and variances homogeneous. All these analyses were performed using the R

206 function *glm*. Specific comparisons were performed using a Tukey post-ANOVA through the R package

207 *multcomp*.

208

209 *Variation partitioning analysis*

210 Fitted models were also used to perform a variation partitioning analysis (Borcard et al. 1992) to assess

211 the relative contribution of *WGP* and *TGP* in each climate region. For this, fitted models were run by

212 population, heat-shock periods (larval data) and sex (adult data). Each acclimation effect was fitted

213 independently as well as combined, and then coefficients of determination were extracted to estimate

214 their relative contribution to total variation using the function *varPart* of R package *modEvA* (Barbosa

215 et al. 2013, 2016).

216

217   *Individual based simulations of WGP and TGP*

218   We used individual-based computer simulations to assess how differences in climatic conditions between

219   Sonora and Catalina affect the long-term evolution of within and transgenerational plasticity (see

220   Appendix S1 in Supporting Information for a more extensive description of parameter values included

221   in the model, and Appendix S2 for analysis of the adaptation of the temperature time series from historical

222   temperature data). Extending previous quantitative genetics models on cascading maternal effects

223   (Kirkpatrick and Lande 1989; Kuijper and Hoyle 2015), we consider a well-mixed population of $N =$

224   10,000 diploid individuals with non-overlapping generations. Individuals are then allowed to adapt to a

225   realistic fluctuating environment as extracted from historical climate data from Catalina and Sonora [Data

226   provided by National Centers for Environmental Information, National Oceanic and Atmospheric

227   Administration   (NOAA)   from   their   web   site   https://www.ncdc.noaa.gov/cdo-

228   web/datasets#NORMAL_HLY (Figure 1)], during 50,000 generations (see Figure S6 for an example

229   simulation), where within and between generational plasticity is allowed to vary between larval and adult

230   individuals. Hence, the phenotype of a larval individual is z_{lv} while the adult phenotype is z_{ad}.

231   Specifically, the larval phenotype $z_{lv,t+\tau_0}$ in generation t at the time of birth τ_0 (where $\tau_i = \frac{i}{\ell}$ is the

232   number of days relative to the total lifespan ℓ measured in days) is given by

233

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

235

236   Here, the larval phenotype $z_{lv,t+\tau_0}$ is affected by three evolving traits, with $a_{t+\tau_0}$ reflecting the genetic

237   basis of the phenotype in the absence of within and transgenerational plasticity, $b_{lv,t+\tau_0}$ reflecting the

238   strength of larval within-generational plasticity in response to the environment experienced at the time

239   of birth $\varepsilon_{t+\tau_0}$ and finally $m_{lv,t+\tau_0}$ reflects the strength of the transgenerational effect that depends on the

240   adult mother's phenotype $z^*_{ad,t-1}$, where the * denotes a phenotype after it experienced survival

241 selection. The variable $e_{t+\tau_0}$ reflects developmental noise, which is a random variable drawn from a

242 normal distribution with mean 0 and variance σ_e^2.

243

244 After birth, a larva with phenotype z, plasticity b and maternal effect m experiences stabilizing mortality

245 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

246 by

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

248

249 where $s_{\min}$ is a baseline survival probability to prevent populations going extinct (as we are interested in

250 the values of m and b that evolve in certain regimes rather than in where and when populations go extinct).

251 Throughout, we assume $s_{\min} = 0.5$. Within the exponential term, we assume that the optimal phenotype

252 (to maximise survival probability) is $\varepsilon_{t+\tau_i}$, the temperature of that day (see Appendix S2 "Adaptation to

253 temperature timeseries"), while ω_z^2 is the width of the selection function, small (large) values of which

254 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

255 generational plasticity and maternal effects respectively (Kuijper and Hoyle 2015).

256

257 Larvae which have survived according to eq. (2) for $\tau_{\text{ad}}\ell$ days become adults, after which they develop

258 an adult phenotype $z_{\text{ad},t+\tau_{\text{ad}}}$ in generation t, where within and transgenerational plasticity of the adult

259 phenotype can evolve independently from the same traits for the larval phenotype. Hence, we have:

260

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

262

263 where $a^*_{t+\tau_{ad}}$ reflects the elevation, which is the same trait as expressed in larvae, conditional on that the

264 individual has survived for $\tau_{ad}\ell$ days (denoted by *). The strength of within-generational plasticity in

265 adulthood is $b^*_{ad,t+\tau_{ad}}$, which reflects the strength of the reaction norm in response to the environment

266 $\varepsilon_{t+\tau_{ad}}$ at the onset of adulthood. Regarding transgenerational plasticity, $m^*_{ad,t+\tau_{ad}}$ reflects sensitivity to

267 the maternal phenotype at adulthood. Here, the maternal phenotype $z^*_{ad,t-1}$ is the same phenotype that

268 was experienced as larva, reflecting, for example, persistent maternally transmitted chromatin

269 modifications, small RNAs or nutrients (Moore et al. 2019) . Finally, $e_{t+\tau_0}$ again reflects developmental

270 noise.

271

272 The traits b_{lv}, b_{ad}, m_{lv} and m_{ad} are each assumed to be coded by single diploid loci, whereas the elevation

273 a is assumed to be coded by 5 diploid loci, in line with previous models where the additive genetic

274 variance in elevation is typically taken to be larger than the additive genetic variance in plasticity (e.g.,

275 Hoyle and Ezard, 2012; Lande, 2009). For the sake of simplicity, all loci are unlinked and evolve

276 according to a continuum of alleles model (Kimura and Crow 1964). The probability that each allele

277 mutates per generation is $\mu = 0.01$, after which a random number drawn from a normal distribution with

278 mean 0 and variance 4×10^{-4} is added to the current allelic value.

279

280

281 **Results**

282

283 Acclimation treatments performed at 36°C (versus 25°C) in parents and F_1 larvae significantly increased

284 tolerance of heat-shocked larvae as measured through viability components (Table 1, Figure 2a), while

285 only within-generation acclimation increased heat knockdown in adults (Table 2, Figure 2a). Unlike

286 viability components, development time did not always increase in response to the acclimation treatments

287 (Table 1, Figure 2a). Larva-pupa and larva-adult components of viability and development time showed

288 significant effects of acclimation treatments and population, whereas the percentage of hatching pupa

289 was not affected (Table S1). Therefore, thermal responses in larva-to-pupa and larva-to-adult were highly

290 correlated (*Viability Spearman's r* = 0.99, *P* < 0.01 and *Development time Spearman's r* = 0.94, *P* <

291 0.01). These results suggested that acclimation treatments performed in larvae only affected the larva-to-

292 pupa transition and not pupa-to-adult.

293

294 *Larval tolerance to upper thermal limits*

295 Viability was analyzed as a response to heat-shocks following acclimation as well as standardized by the

296 control treatments (acclimation treatments without being heat-shocked) (Table 1). Standardized viability

297 was used to confirm whether detected responses to heat shocks persist after controlling for acclimation

298 effects on non-heat-shocked larvae. Population, heat-shock periods, parental and F_1 larval acclimation

299 treatments were significant for both viability and standardized viability (Table 1). Longer heat-shock

300 periods lead to lower viability (see Figure 2a and for results at 1.5 and 2h heat shock) but tended to

301 increase population and acclimation effects. Hereafter we focus on results obtained in for 2h heat shock

302 in larvae (Figure 2a). All acclimation treatments increased heat tolerance, but several paired interactions

303 were detected for viability, showing differential effects of *WGP* and *TGP* according to population, heat-

304 shock period as well as interactions between acclimation treatments ($Accl_{larva}*Accl_{parents}$) (Table 1).

305 Most of these interactions were not significant for standardized viability, except for the $Pop*Accl_{larva}$

306 and $Pop*Accl_{parents}*Accl_{larva}$ interactions (Table 1), indicating that the level of *WGP* and *TGP* were

307 different between populations (Figure 2a). The Sonoran population exhibited the largest plastic

308 responses, and these effects were more evident from combinations of treatments where both parents and

309 F_1 larvae were acclimated 36°C (*matched* cues), increasing heat tolerance by up to 63% when compared

310 to *mismatched* cues (Figure 2a). In contrast Catalina had higher plastic responses when only one of the

311　generations was acclimated, which increased their thermal performance by up to 45% (*mismatched* cues)

312　when compared to *matched* cues (Figure 2a).

313

314　Only population and F_1 larval acclimation affected components of development time as main effects,

315　while the heat-shock period did not nor did any of its interactions. However, there were complex paired

316　interactions indicating differences in the effect of parental and F_1 larval acclimation between populations

317　as well as interactions between acclimation treatments ($Accl_{larva}*Accl_{parents}$) (Table 1). The triple

318　interaction $Pop*Accl_{parents}*Accl_{larva}$ (Table 1) indicated a complex pattern in which Catalina exhibits

319　positive *WGP*, but negative *TGP*, while the Sonoran population exhibits positive effects for both

320　acclimation treatments (Figure 2a). Moreover, Catalina only showed *WGP* for larvae coming from

321　untreated parents (*mismatched* cues), increasing development time by up to nearly two days, while no

322　larval acclimation was detected as *TGP* (Table 1, Figure 2a). For the Sonoran population, the pattern was

323　opposed to that in Catalina, both *WGP* and *TGP* were positive, increasing development time in over two

324　days. As for viability data, these effects were much larger when both parents and F_1 larvae were

325　acclimated at 36°C (*matched* cues) (Figure 2a).

326

327　*Adult tolerance to upper thermal limits*

328　Thermal tolerance in adults was measured as heat-knockdown time during ramping heat shocks in

329　response to acclimation treatments performed in parents, F_1 larvae and F_1 adults. Neither the temperature

330　experienced by parents (Table 2) nor acclimation performed in F_1 larvae affected heat knockdown in F_1

331　adults or any of their interactions (Table S2), so these effects were removed from the final model (Table

332　2). Acclimation performed in F_1 adults significantly increased heat knockdown (Table 2, Figure 2a), but

333　the response differed between populations and sexes (Table 2, Figure 2a). Two interaction effects were

334　detected (Table 2), suggesting that the level of acclimation performs differently between populations (

335   *Pop*Accl_{adults}*) and sexes (*Accl_{adults}*Sex*), being higher in Sonoran females, as their heat-knockdown

336   time increased by over 20 min, while it was increased by nearly 10 min in Catalina (Figure 2a).

337

338   *Variation partitioning analysis*

339   Relative contributions of *WGP* and *TGP* to thermal tolerance as estimated from fitted models indicated

340   that adults not only did not express *TGP*, but had the lowest *WGP* component (14% in Sonora) when

341   compared to that in larvae (viability = 39%, development time = 19% in Sonora) (Figure 2b). The *WGP*

342   component of larval tolerance was higher in Sonora for both viability (39%) and development time (7%)

343   (Figure 2b). The *TGP* component was also higher for the Sonoran population, at 17%, while it explained

344   only 10% of variation in the population of Catalina (Figure 2b). Finally, the *TGP* component of

345   development time explained 13% of phenotypic variation in Catalina, while the Sonoran population only

346   exhibited 3% (Figure 2b). However, this variation in Catalina was associated with *TGP* decreasing

347   development time in this population (Figure 2b) as opposed to Sonora.

348

349   *Individual-based simulations of within and transgenerational plasticity*

350   Simulated values of *WGP* and *TGP* (Figures S7 and S8) were obtained for larvae and adults under

351   different scenarios of plasticity and selection costs (see Table S3 for simulation parameters) in

352   simulations corresponding to the same experiment as performed in the laboratory (Appendix S1), with

353   parental and F_1 offspring environments (25 vs 36°C). Since the model does not consider direct

354   interactions between populations and/or plastic responses, expectations for empirically detected

355   interactions cannot be detected from plots of *match*/*mismatch* cues. Simulated data are more likely to be

356   strictly adaptive rather than exhibit short-term carry-over effects that can generate the observed

357   interactions (Kuijper and Hoyle 2015).

358

359 Simulated larva and adult stages evolving under a Sonoran regimen resulted in higher levels of adaptive

360 *WGP* and *TGP* than those in Catalina (Figure 3), mimicking the main findings from the experimental

361 evidence in all traits analyzed (Figure 2a). Viability results indeed are in line with simulated plastic

362 responses while developmental time showed a negative *TGP* in Catalina (Figure 2a) which was not

363 obtained from simulations (Figure 3a), but the positive value of the trait was still higher in Sonora. Adult

364 heat knockdown tolerance supported the expectation of adaptive tolerance to upper thermal limits as

365 observed from the simulations (Figure 3b), while there was no *TGP* in adults detected in the empirical

366 data (Figure 2a). We found that the prediction of stronger *TGP* and *WGP* in Sonora is robust to varying

367 the strength of fluctuating stabilizing selection (Figures S7 and S9) or varying the cost of phenotypic

368 plasticity (Figures S8 and S10). Similarly, we find that adaptive *TGP* is generally stronger when affecting

369 larval rather than adult traits (Figure 3 and S7, S8), again in line with empirical findings of viability and

370 heat knockdown traits (Figure 2). Adaptive *WGP* on the other hand was expected to be higher for adult

371 traits in simulated data (Figures S7 and S8) as opposed to empirical findings (Figure 2a), where *WGP*

372 was clearly higher in larval traits. This result suggests additional constraints missing from our model

373 when considering developmental stages with different reproduction costs (larval vs adult). Our model

374 suggests that realistic fluctuations in temperature can explain the differential evolution of *TGP* and *WGP*

375 across climatic regions.

376

377

378 **Discussion**

379

380 By combining experimental evidence with individual-based simulations of phenotypic plasticity over

381 generations, we were able to disentangle the adaptive significance of thermal plasticity across life stages

382 in an ecological context with substantial climate variability in the desert *D. mojavensis*. We demonstrated

383   that the level of variation and environmental predictability can shape tolerance to upper thermal limits

384   within and between generations and that *TGP* evolves when the parental environment is a good predictor

385   of that experienced by the offspring. *WGP* was higher in larvae than adults, while *TGP* was only detected

386   in larval stages. Although both regional climates showed significant plastic responses, the population

387   from the Sonoran Desert, evolving under high thermal variability relative to that of Mediterranean

388   climate in Catalina Island (Figures 1b and S5) led to increased plasticity when both parents and offspring

389   were acclimated (*matched* cues). The combined analysis of empirical and simulated data suggested that

390   life stage and regional variation of thermal *WGP* and *TGP* is adaptive in *D. mojavensis*.

391

392   *Within-generation plasticity*

393   Acclimation performed within generations significantly increased heat tolerance in both larvae and

394   adults, although this was only evident when acclimation was conducted in the same developmental stage,

395   moreover acclimation treatments performed in larvae did not affect tolerance in adults. As expected from

396   a costly temporal response (Krebs and Loeschcke 1994; Dahlhoff and Rank 2007), this result

397   demonstrates that acclimation, as performed through a brief exposure to an environmental cue, does not

398   provide hardening against subsequent heat-shocks occurring in the long term. However, this acclimation

399   still affected later larval stages, as evident from the pronounced effect that acclimated larvae had on

400   development time. Changes detected in development time are likely a consequence of the cost associated

401   with the heat shock response in each population. This acclimation effect commonly known as heat

402   hardening, has been widely detected across several species for decades (Hoffmann et al. 2003; Sgrò et

403   al. 2010; Kellermann and Sgrò 2018), even in *D. mojavensis* (Krebs 1999; Krebs and Bettencourt 1999).

404   Heat hardening is mainly caused by rapid expression of heat-shock proteins (HSPs) and other molecular

405   components that protect denatured proteins and tissues from damage caused by high thermal exposures

406   (Dahlgaard et al. 1998; Bahrndorff et al. 2010; Diaz et al. 2015; Cai et al. 2017). These components are

407   known to accumulate rapidly during mid-range temperatures (*e.g.* 36°C) as occurs in *D. mojavensis*

408   (Krebs 1999; Krebs and Bettencourt 1999).

409

410   We observed that *WGP* had a higher contribution to larval tolerance when compared to adult tolerance

411   based on variation partitioning. This is consistent with literature on thermal tolerance in several

412   organisms, reporting a greater thermal resistance at early life stages when compared to adults (Sørensen

413   and Loeschcke 2002; Zizzari and Ellers 2014). Early stages including larva, are more bound to the

414   fluctuations of their environment since they are constrained to their substrate, while flying adults can

415   seek more suitable thermal microclimates (Krebs and Loeschcke 1995; Feder et al. 1997). Moreover, the

416   molecular machinery of heat-shock response is known to involve considerable energy cost (Krebs and

417   Loeschcke 1994; Dahlhoff and Rank 2007), which often leads to trade-offs between life stages and

418   reproductive-related behaviors (Jørgensen et al. 2006; Zhang et al. 2015) leading to more limited *WGP*

419   in adults (Sørensen and Loeschcke 2002) as has been previously found in *D. mojavensis* (Patton et al.

420   2001; Fasolo and Krebs 2004).

421

422   *Transgenerational plasticity*

423   We detected *TGP* only for larval tolerance, where acclimated parents led to larvae that were more

424   resistant to upper thermal limits. The parental acclimation had an opposed effect on development time

425   of Catalina vs Sonora, increasing development time in Sonora but decreasing in Catalina. This result

426   suggests potential costs on development associated with *TGP* in Sonora and supports the major role of

427   plastic responses in early stages discussed above for *WGP*. Unlike *WGP*, inferring the adaptive

428   significance of *TGP* is more challenging. Despite the recent interest in non-genetically inherited effects

429   and their role in evolution (Mousseau and Fox 1998; Galloway and Etterson 2007; Bonduriansky et al.

430   2012; Nestler 2016), more particularly for climate change scenarios (Burgess and Marshall 2011;

431   Münzbergová and Hadincová 2017; Bonamour et al. 2019), little attention has been paid to formally

432   testing their adaptive significance. As suggested by Donelson et al. (2018) and Uller et al. (2013), these

433   effects are often negative, neutral (Sikkink et al. 2014) or comparatively much weaker than *WGP*. The

434   observed positive *TGP* could still be a simple non-adaptive carry-over effect, a consequence of stressed

435   embryos during parental acclimation or a *silver spoon* effect (Engqvist and Reinhold 2016; Sheriff et al.

436   2018). A more formal link to the adaptive significance of these effects should be investigated in relation

437   to the predictability of environmental variation while accounting for the life cycle of the target species

438   (Bonamour et al. 2019). Based on this premise, we investigated the effect of parent-offspring

439   predictability of climatic variation over time on the evolution of simulated *TGP* and *WGP* in a realistic

440   environment (Figures S6-S10). Our simulated data indicated that *TGP* on larval traits is stronger because

441   the parental phenotype is more likely to predict the environment experienced by its offspring during their

442   larval stage, which strongly suggest that *TGP* is likely to be adaptive in larvae. The environment is more

443   likely to have changed when offspring are adults.

444

445   Surprisingly, although to a lesser extent, our simulations also predicted *TGP* for adults. The absence of

446   *TGP* in our empirical adult data as opposed to simulated data suggests that the brief environmental cue

447   used to treat parents may not be strong enough to trigger a plastic response between adult generations.

448   However, the parent-offspring predictability included in the simulated data suggests potential effects for

449   longer cues, such as for example when individuals are exposed to environmental cues during a great part

450   of or whole life cycle, a prediction that remains to be formally tested. Qualitative differences between

451   larvae and adults are also expected from the major role played by maternal molecular factors in early

452   stages before hatching larva (Tadros and Lipshitz 2009). This is more related to the limited transcriptional

453   capacity of *Drosophila* embryos as for other oviparous ectotherms, being highly dependent on maternal

454   factors in comparison to later stages, which makes them particularly sensitive to thermal exposure

455   (Walter et al. 1990). Maternal oogenesis establishes the early embryonic transcriptome and proteome

456   (Schüpbach and Wieschaus 1986; Wieschaus 1996; Tadros and Lipshitz 2009), which are therefore major

457   determinates of embryo fitness. Recently Lockwood et al. (2017) have found molecular evidence that

458   demonstrates a positive effect of small heat-shock proteins from maternal ovaries on the thermal

459   performance of embryos in *D. melanogaster*. This fact offers an additional selection pressure for maternal

460   effects on early stages, particularly for recently hatched larvae that can potentially carry over a great load

461   of these maternal factors.

462

463   *Adaptive significance of WGP and TGP is related to regional climate*

464   The environment of the Sonoran Desert exhibits more climatic variability compared to the Mediterranean

465   and buffered climate of Catalina Island and was therefore predicted to express higher plastic responses

466   (Figures 1b and S5). Except for adult data (*TGP* not detected for heat knockdown), all traits analyzed

467   exhibited regional variation. For larval tolerance, variation partitioning analysis evidenced greater

468   relative components of *WGP* and *TGP* in the Sonoran region when compared to those in Catalina.

469   Overall, this result agreed with our expectations of adaptive plasticity between climatic regions based on

470   simulated data, without considering interaction effects. Furthermore, we detected that plasticity effects

471   were condition-dependent between generations, with Sonora exhibiting the most pronounced plasticity

472   when both parents and offspring were acclimated (*matched* cues). When only one generation was

473   acclimated (*mismatched* cues), the population from Catalina showed either similar or greater effects than

474   Sonora. These results are consistent with theoretical considerations for adaptive significance of *TGP*

475   (Uller et al. 2013). When parental acclimation is adaptive, it is expected to increase tolerance of the next

476   generation while minimizing costs associated with physiological or molecular mechanisms of tolerance

477   (e.g. heat-shock response (Krebs and Loeschcke 1994; Dahlhoff and Rank 2007)). These carry-over

478   effects would generate trade-offs with detriment to offspring fitness when their environment does not

479 resemble the parental experience (Uller et al. 2013; Sheriff et al. 2018), suggesting that mechanisms of

480 plasticity in response to environmental stress are preferentially triggered under *matching* cues compared

481 to *mismatched* cues, i.e. "adaptive matching" following Uller et al. (2013).

482

483 Given that the *match/mismatch* framework has been recently challenged by Engqvist and Reinhold

484 (Engqvist and Reinhold 2016), here we have provided an alternative approach to infer the adaptability

485 of *TGP*, by using long-term evolutionary simulations of *WGP* and *TGP* under realistic scenarios extracted

486 from historical climate data. We found that predictability and amplitude of temperature fluctuations are

487 larger in Sonora than in Catalina (Figures S2, S3, S4 and S5), suggesting stronger selection on both *WGP*

488 and *TGP* in the Sonoran Desert relative to Mediterranean climate in Catalina.

489

490 Expectations for empirically detected interactions between populations and plasticity of thermal

491 tolerance are not possible to simulate directly, since available models don't consider direct interactions

492 between plastic responses. However, since the simulations specifically involve adaptive evolution of

493 *WGP* and *TGP*, these are strictly adaptive changes rather than carry-over effects (Kuijper and Hoyle

494 2015). Simulated data are then more likely to be associated with thermal plasticity responses in *matched*

495 acclimation treatments. When *TGP* was detected in larval traits, *matched* acclimation treatments between

496 parents and offspring increased thermal performance in both populations in a higher proportion than that

497 in *mismatched* treatments, which suggests that both populations exhibit adaptive components of plastic

498 responses. However, the Sonoran region expressed the highest plasticity under *matched* acclimation

499 treatments, while exhibiting the lowest response under *mismatched* treatments between generations. This

500 result strongly suggests that *TGP* of tolerance to upper thermal limits exhibit a more predictive

501 component in the Sonoran population, while Catalina seems to express higher unpredictive positive

502 carry-over effects.

503   *Limitations*

504   A common bias in *TGP* estimations involving stress responses is the potential effect that suboptimal or

505   stressful conditions can impose on experimental groups, particularly for early developmental stages

506   (Kaufmann et al. 2014; Heckwolf et al. 2018). The vulnerability of early stages is not always visible and

507   might impose selection pressure for more tolerant genotypes, resulting in a biased estimation of plasticity

508   (Santos et al. 2019). Our approach accounted for such potential bias by acclimating the parental

509   generation as adults. *Drosophila mojavensis* adults have been previously shown to survive temporary

510   exposures to 36°C, both in the lab (Schnebel and Grossfield 1984, 1986; Patton et al. 2001; Krebs and

511   Thompson 2005) and during summertime (Gibbs et al. 2003). Our estimations of *TGP* therefore did not

512   involve differential mortality between experimental conditions and are therefore unbiased. The same

513   rationale applies for our estimations of *WGP* in adults, but potentially not for larval tolerance. Although

514   we controlled for selection on larval tolerance by choosing a suboptimal temperature that *D. mojavensis*

515   larvae tolerated, it was only partially accounted for in eggs. Larval acclimation involved the latter part

516   of egg-to-larva development, and this transition may have been potentially affected by thermal selection.

517   This effect has recently been demonstrated for *ADH* activity (Santos et al. 2019). Our estimations of

518   *WGP* for larval tolerance should be taken with caution since potentially its measurement could have been

519   biased. This means that estimations of *WGP* for larval tolerance may be overestimated in Catalina since

520   this population is presumably more sensitive to thermal conditions compared to Sonora.

521

522

523   **Conclusions**

524

525   To date, the only established framework to infer the adaptive significance of phenotypic plasticity across

526   generations is based on *match*/*mismatch* experiments (Uller et al. 2013). Such an approach has been

527 recently argued (Engqvist and Reinhold 2016) as being insufficient to disentangle adaptive and predictive

528 transgenerational effects from mere carry-over effects or *silver spoons* in certain conditions. Here we

529 propose a more efficient framework by combining the *match*/*mismatch* approach with more recently

530 available models to perform long-term evolutionary simulations of *WGP* and *TGP* (Kuijper and Hoyle

531 2015). As previously suggested, environmental predictability is essential to adaptive *TGP*, and we

532 proposed to account for ecological meaningful environmental variability to perform a more realistic set

533 of simulations that can efficiently help to disentangle such effects. Our proposed framework proved to

534 be highly effective to disentangle strictly adaptive and predictive plasticity across generations as the more

535 likely evolved effect explaining tolerance to upper thermal limits in *D. mojavensis* across life stages in

536 an ecological context with substantial regional climate variability. The proposed framework opens the

537 door not only to study ecological scenarios, but also to extend its application to other avenues of research

538 such as experimental evolution studies to detect qualitatively different levels of both *WGP* and *TGP*.

539

540

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542

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757 **SUPPORTING INFORMATION**

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

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

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

762 **Appendix S3 [**Dataset generated in the current study**]**

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

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

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

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

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

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

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

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

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

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

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

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

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

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788   **TABLES**

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790   **Table 1.** *GLM* analysis for thermal responses (components of viability, standardized viability and
791   development time) following heat shocks after F_1 larval acclimation (*WGP*) and parental treatments
792   (*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
*Pop * HS * Accl_parents * Accl_larva*	1	154	0.262	0.336	154	0.195	0.245	108	0.401	0.350

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

795   LP: larva-pupa

796   LA: larva-adult

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810   **Table 2.** *GLM* analysis for heat knockdown after F_1 acclimation (larvae and adults) (*WGP*) and parental
811   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**
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

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813   Significant values (*p < 0.05*) are highlighted in bold
814   Interactions involving $Accl_{larva}$ were not significant and were not included for simplification (Table 2S).
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831   **FIGURE CAPTIONS**

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833   **Figure 1.** *D. mojavensis* distribution across climatic regions with substantial differences in temperature
834   variability (Desert vs Mediterranean climates). a) Map showing *D. mojavensis* distribution in Santa
835   Catalina Island and Sonoran Desert. b) Daily and seasonal variation of temperature experienced by
836   sampled regions in Catalina and Sonora during 2010 (Data provided by National Centers for
837   Environmental   Information,   NOAA   from   their   web   site   https://www.ncdc.noaa.gov/cdo-
838   web/datasets#NORMAL_HLY). c) Factorial design used to investigate the effect of acclimation as
839   performed at either 25 or 36°C for 24h in parents and *F1* offspring on tolerance to upper thermal limits.

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841   **Figure 2.** Heat-shock tolerance of *D. mojavensis* populations (Catalina vs Sonora) following acclimation
842   treatments performed in parents and *F1* offspring. Heat shocks were performed using a ramping treatment
843   (30°C to 40°C at 0.13°C/min) followed by 2h at 40°C for experiments in larvae or until reaching
844   knockdown for experiments in adult females. a) Results obtained for viability larva-adult (standardized),
845   development time larva-adult and heat knockdown (± *SE*). b) Results of variation partitioning analysis
846   showing the proportion of variation explained by within- (*WGP*) and transgenerational plasticity (*TGP*)
847   for each trait. Only results for 2h heat-shocks in larvae and adult females are shown. Results for 1.5h
848   heat-shocks and adult males are shown in Figure S1.

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850   **Figure 3.** Individual-based simulations showing evolved values of reaction norm slopes (± *SD*) and
851   maternal effects expressed in a) larvae and b) adults. The model predicts that populations from Sonora
852   have evolved both stronger WGP and TGP (at least in larval traits) relative to populations in Catalina,
853   mimicking the empirical findings (Figure 2a). Evolved reaction norms (15 replicate simulations) are then
854   used   to   simulate   the   temperature   exposure   experiment   (Appendix   S2).   Parameters:
855   $\omega\_z^2=\omega\_b^2=\omega\_m^2=10$, $\sigma\_e^2=0.1$, s_min=0.5. The remaining used parameters are in Table S3.

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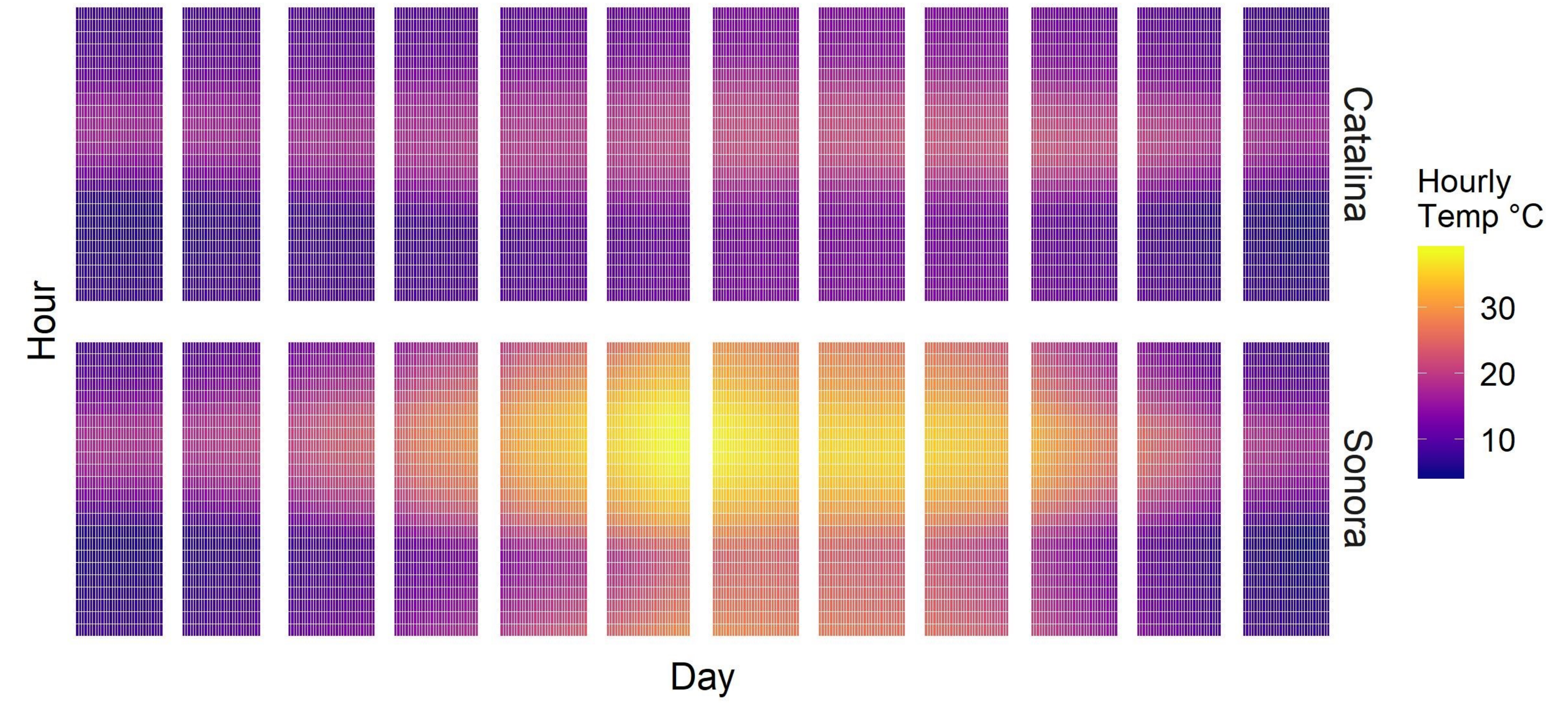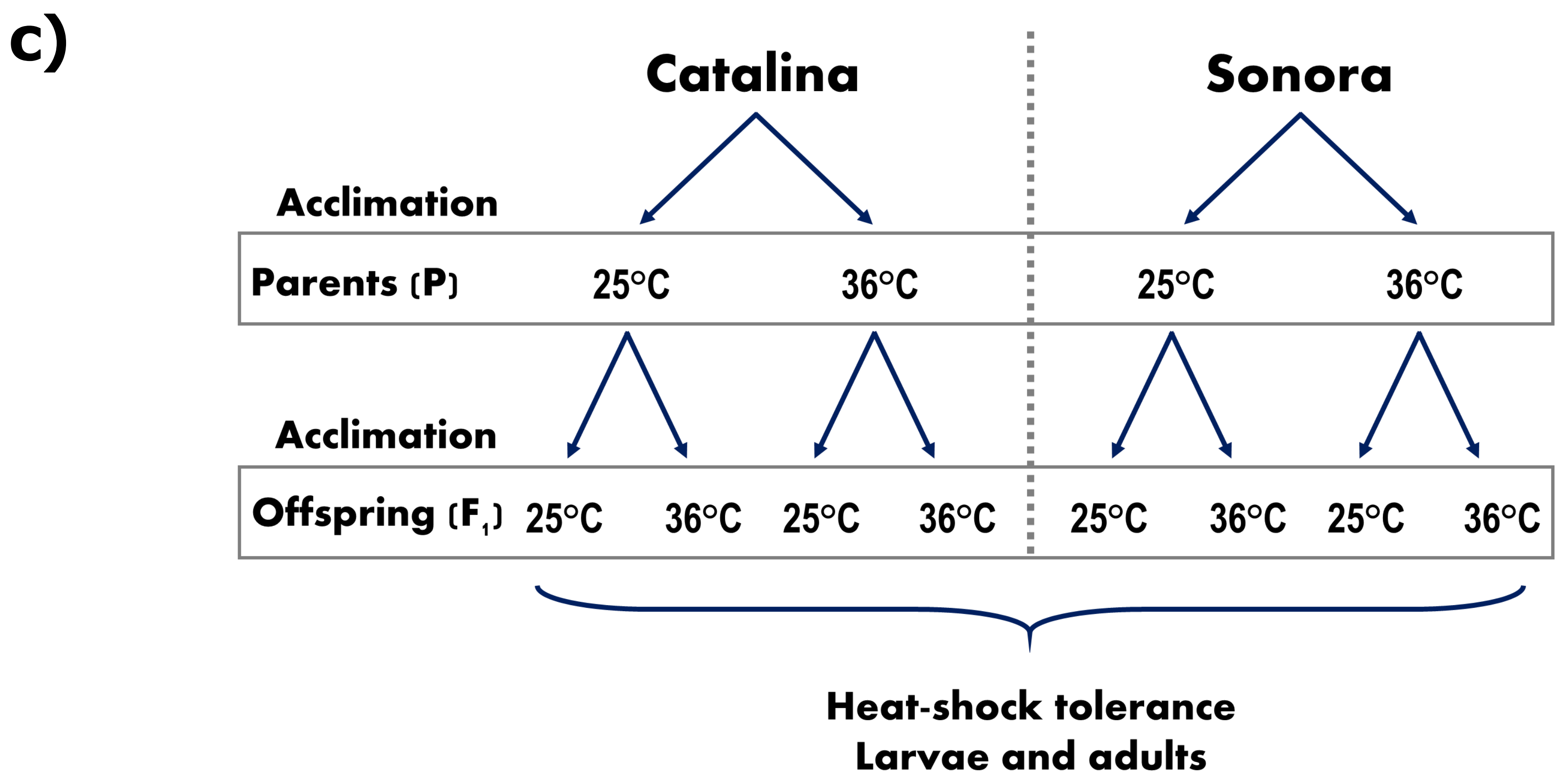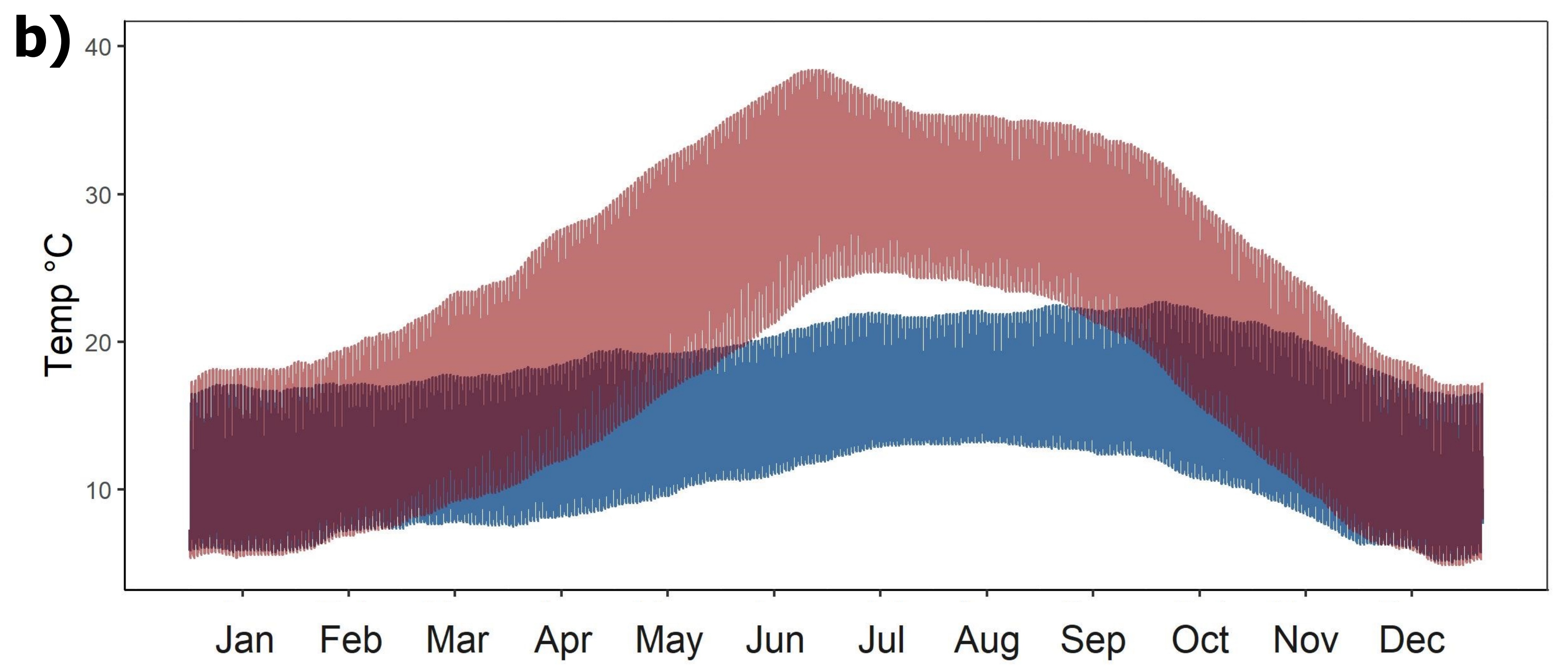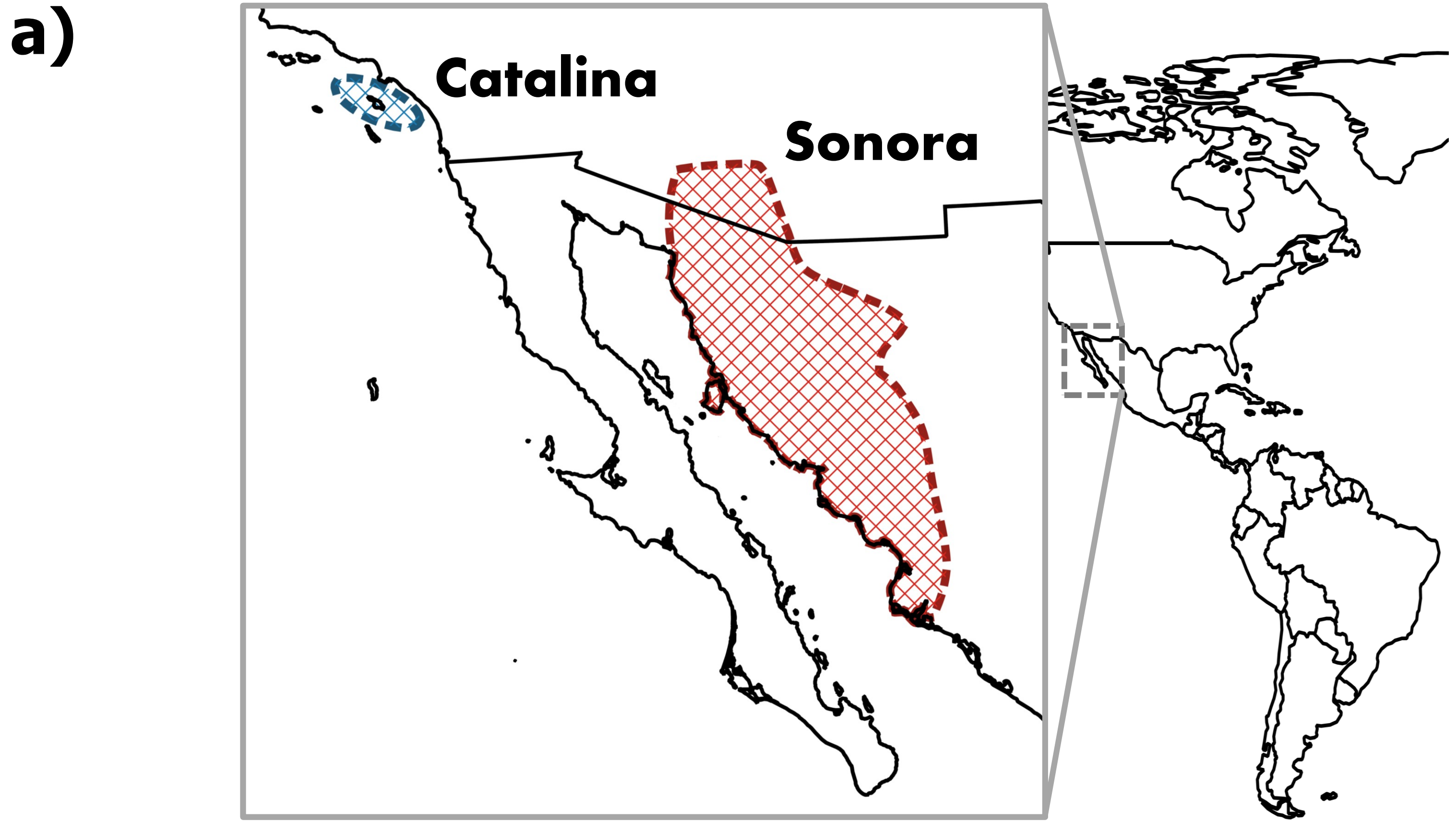
a) *Larva*
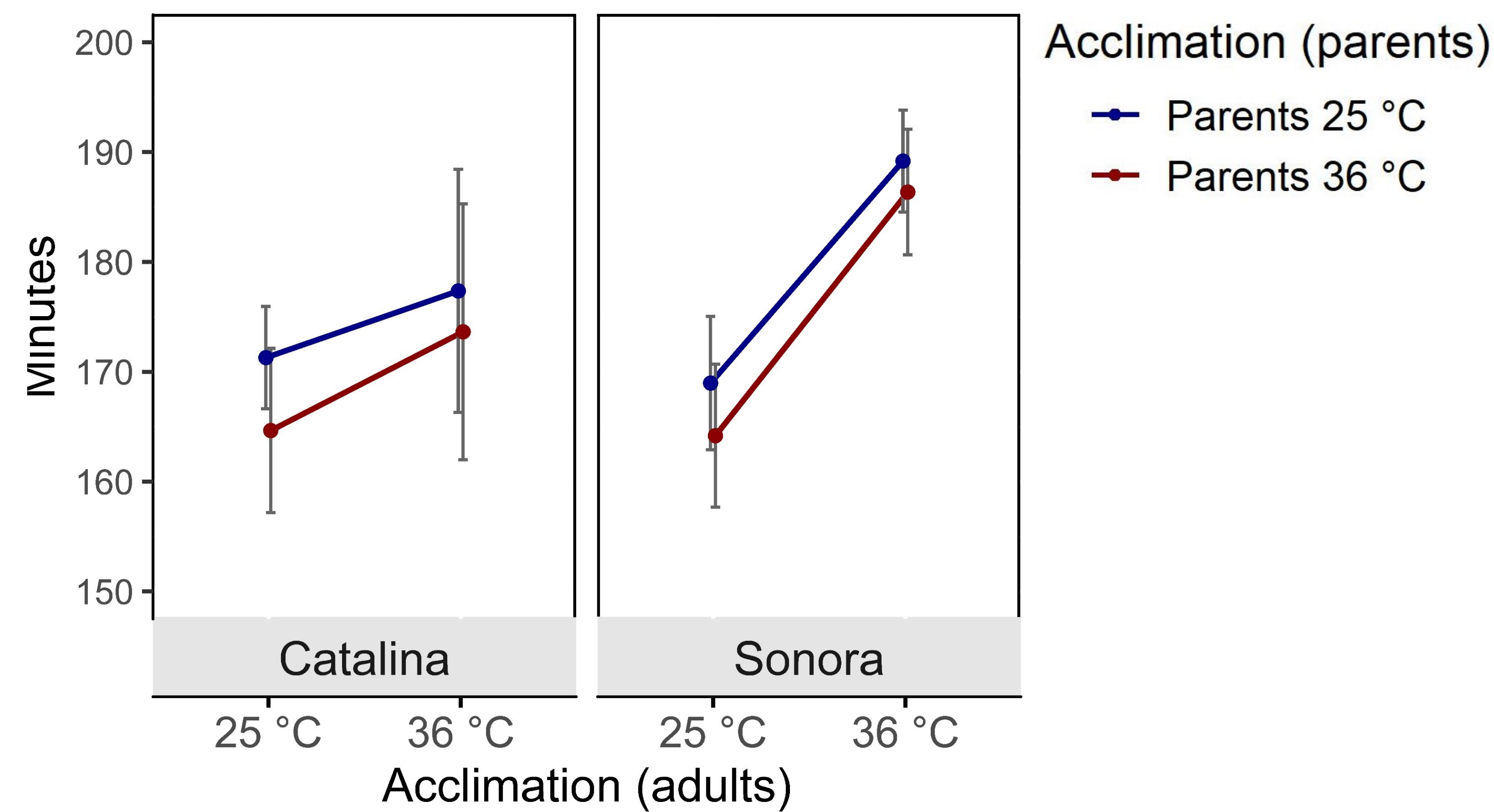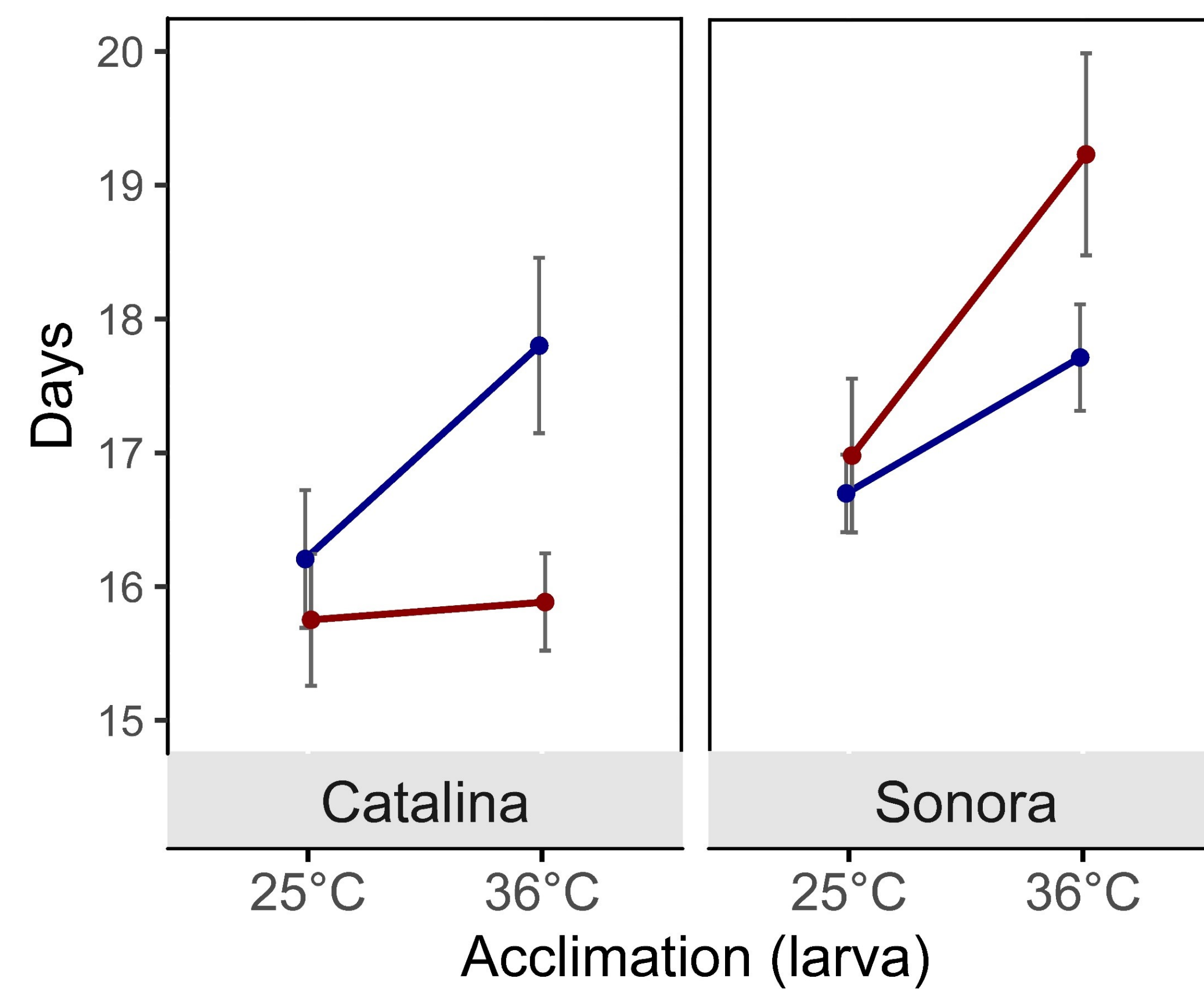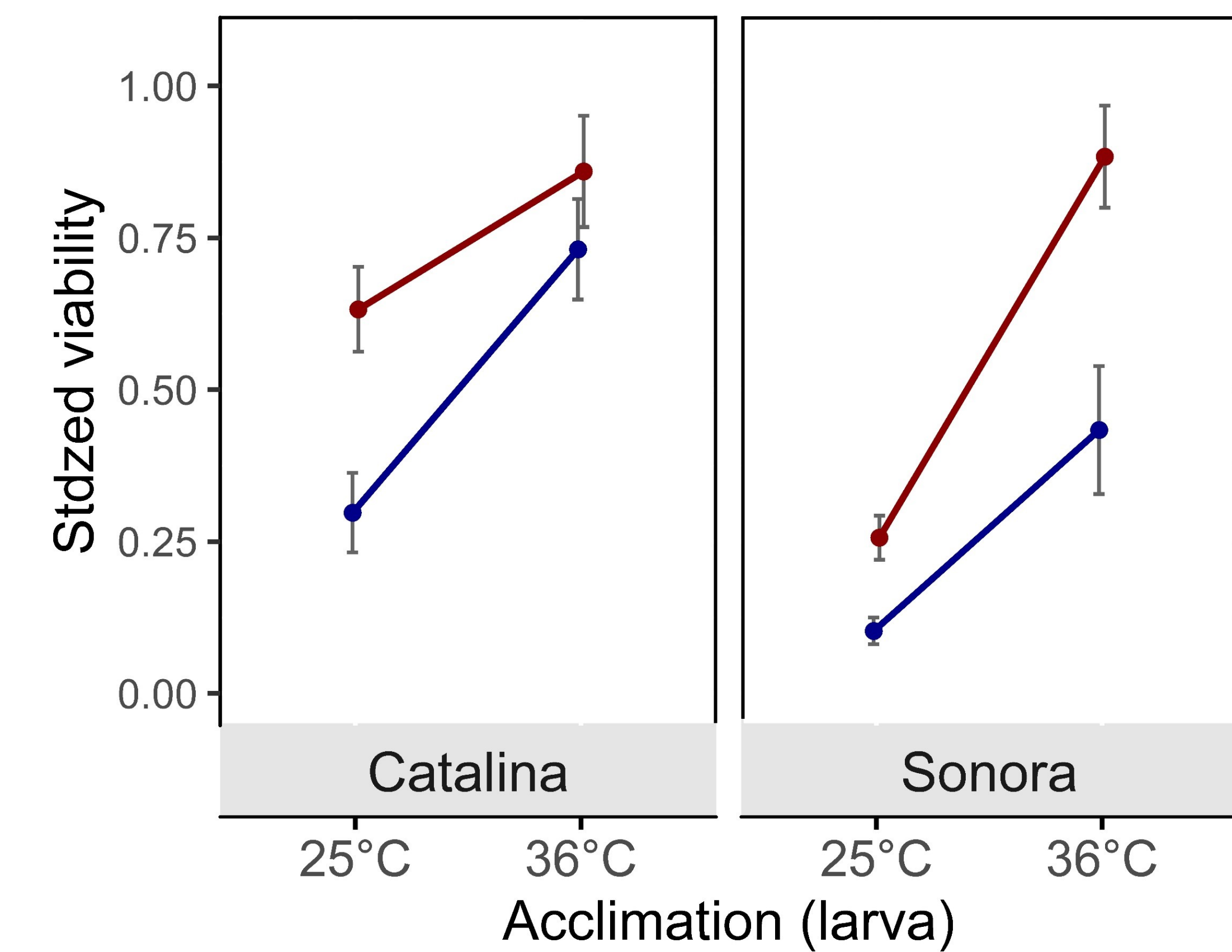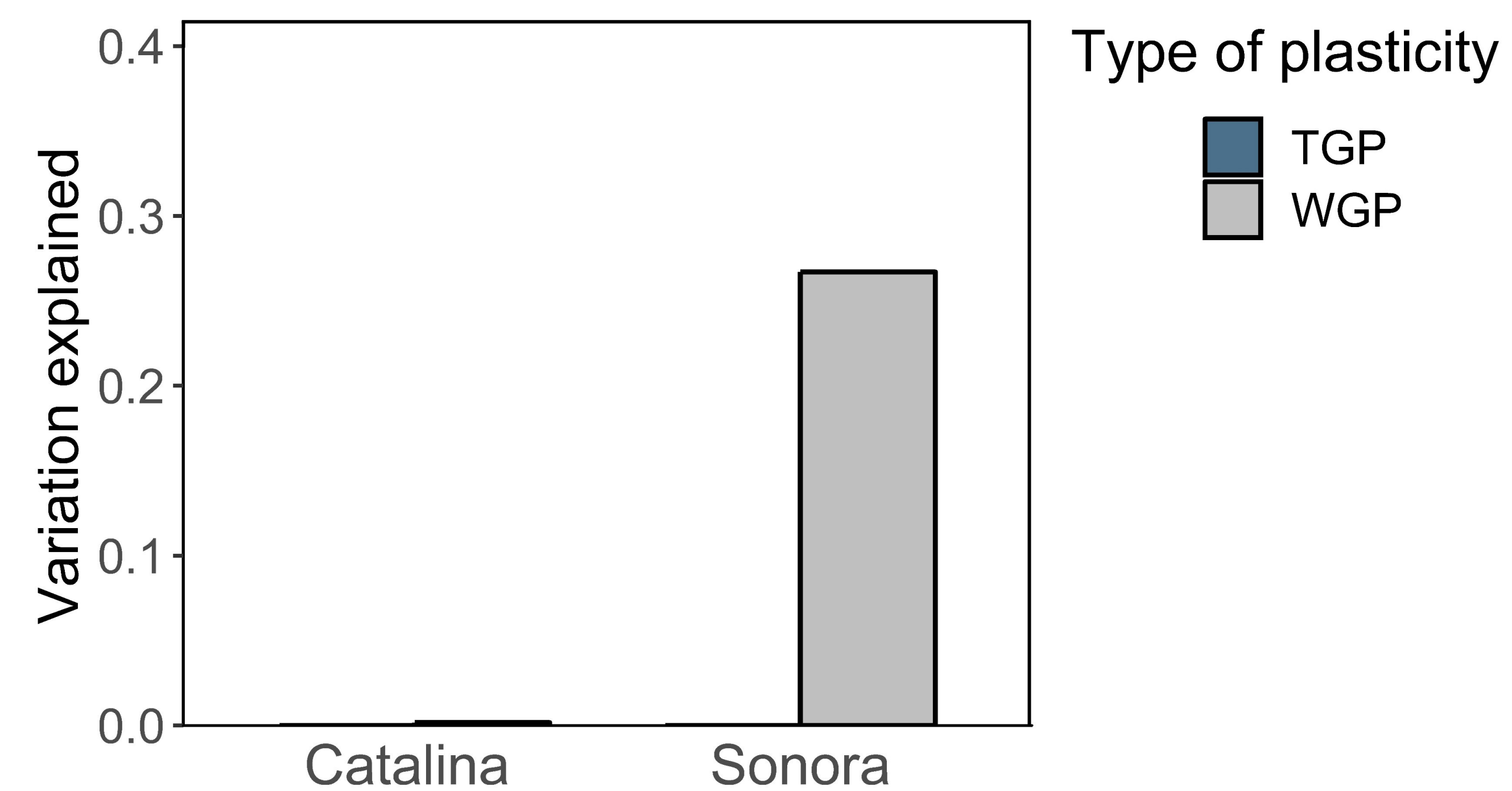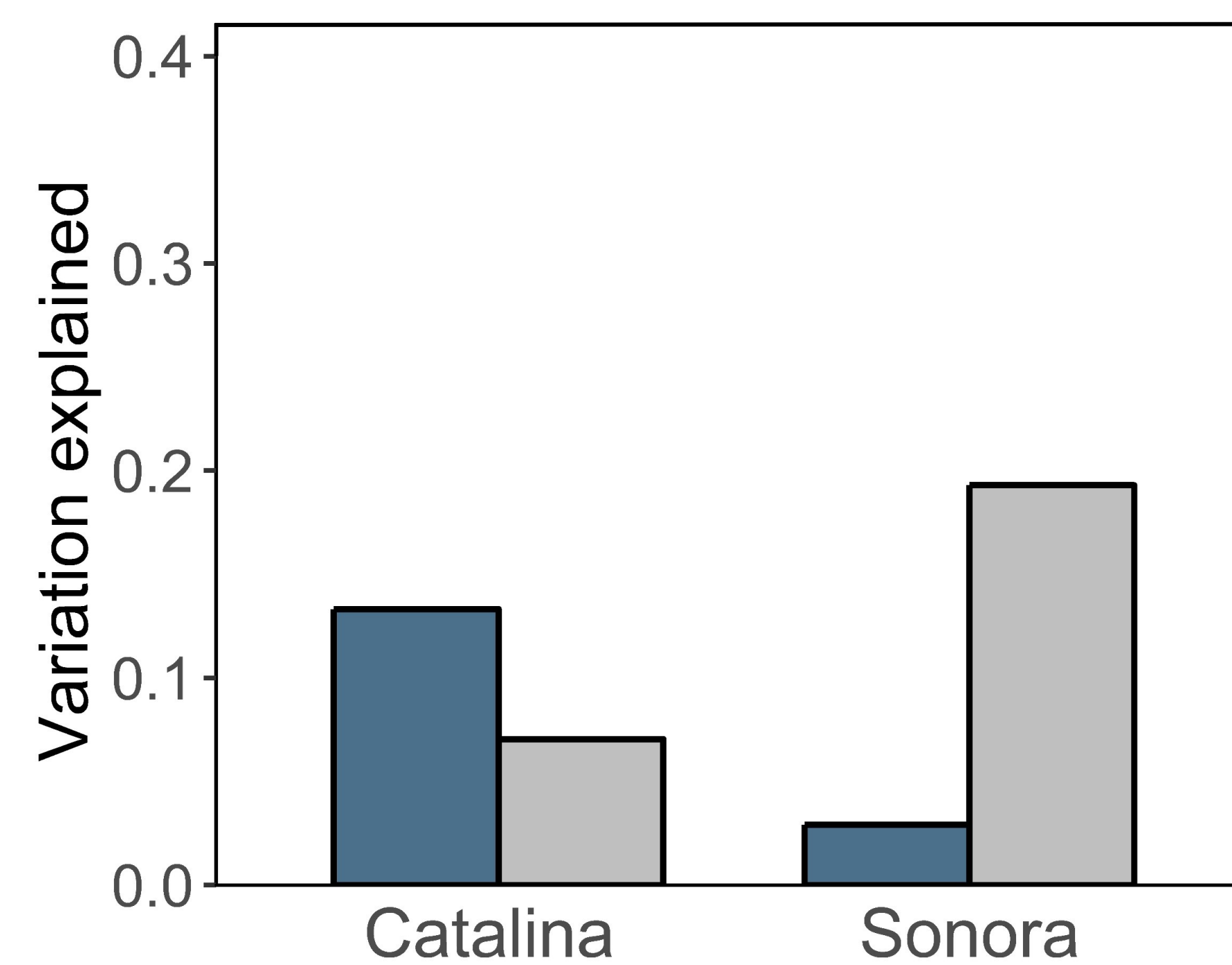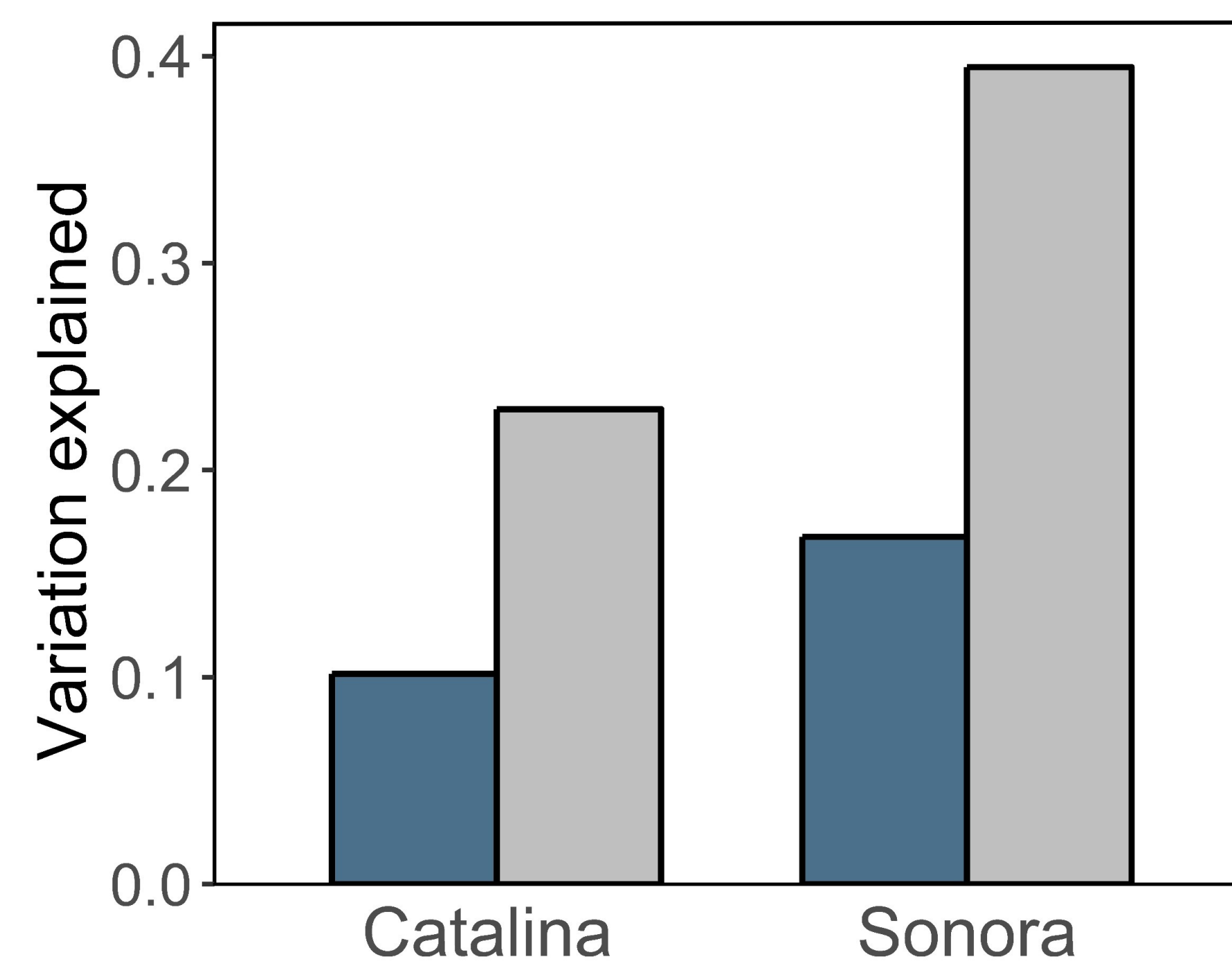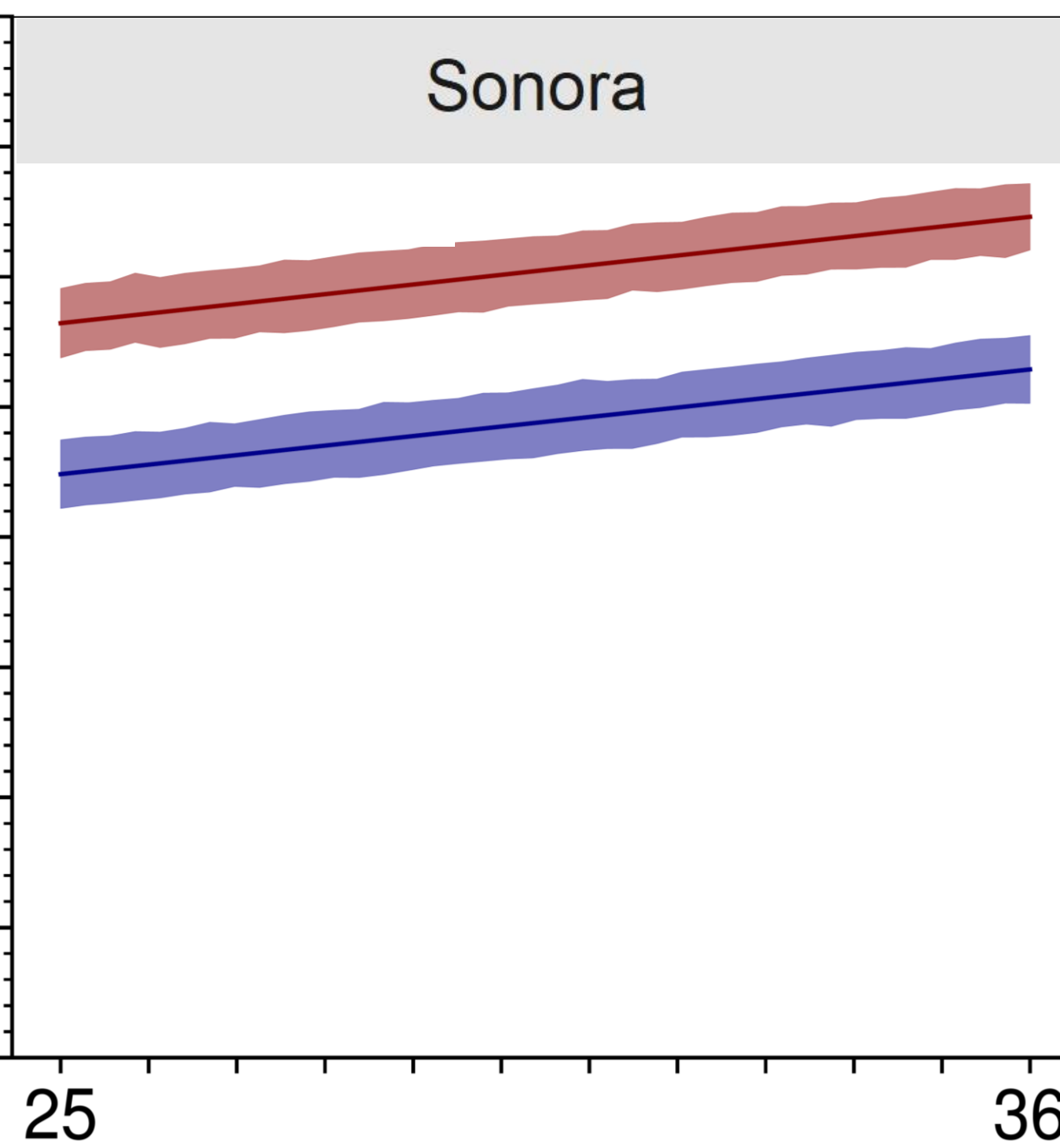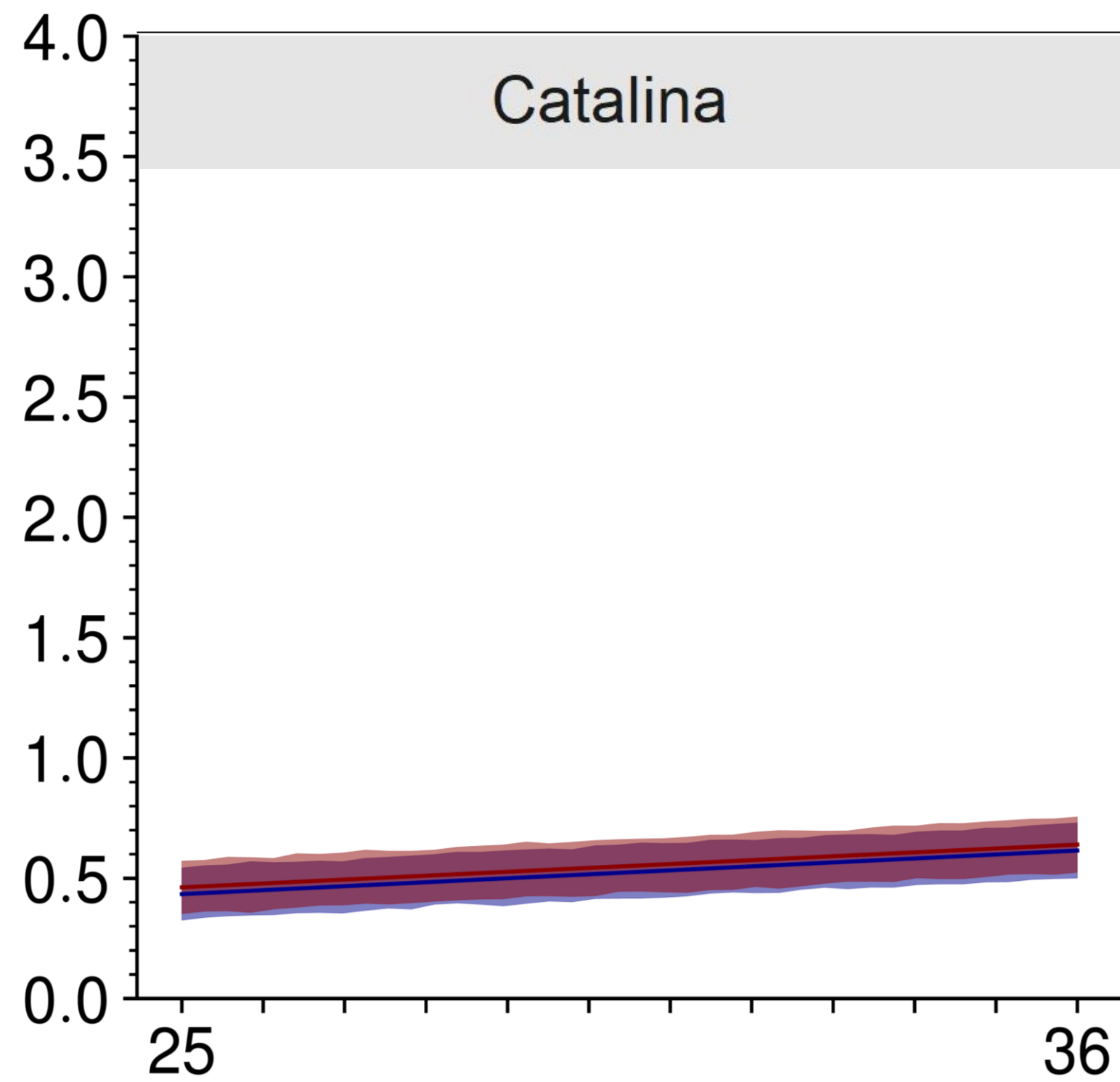
b) *Adults*
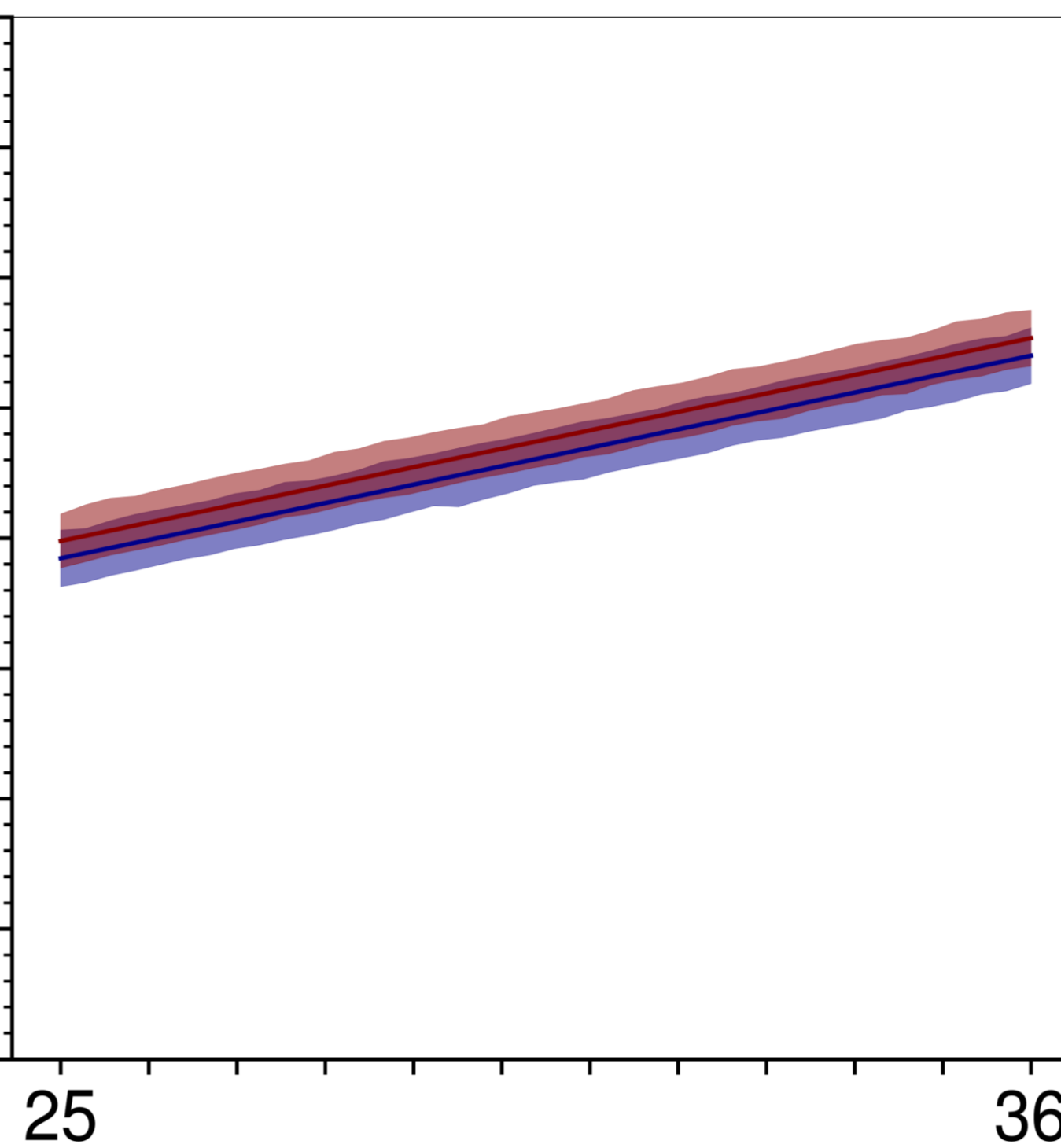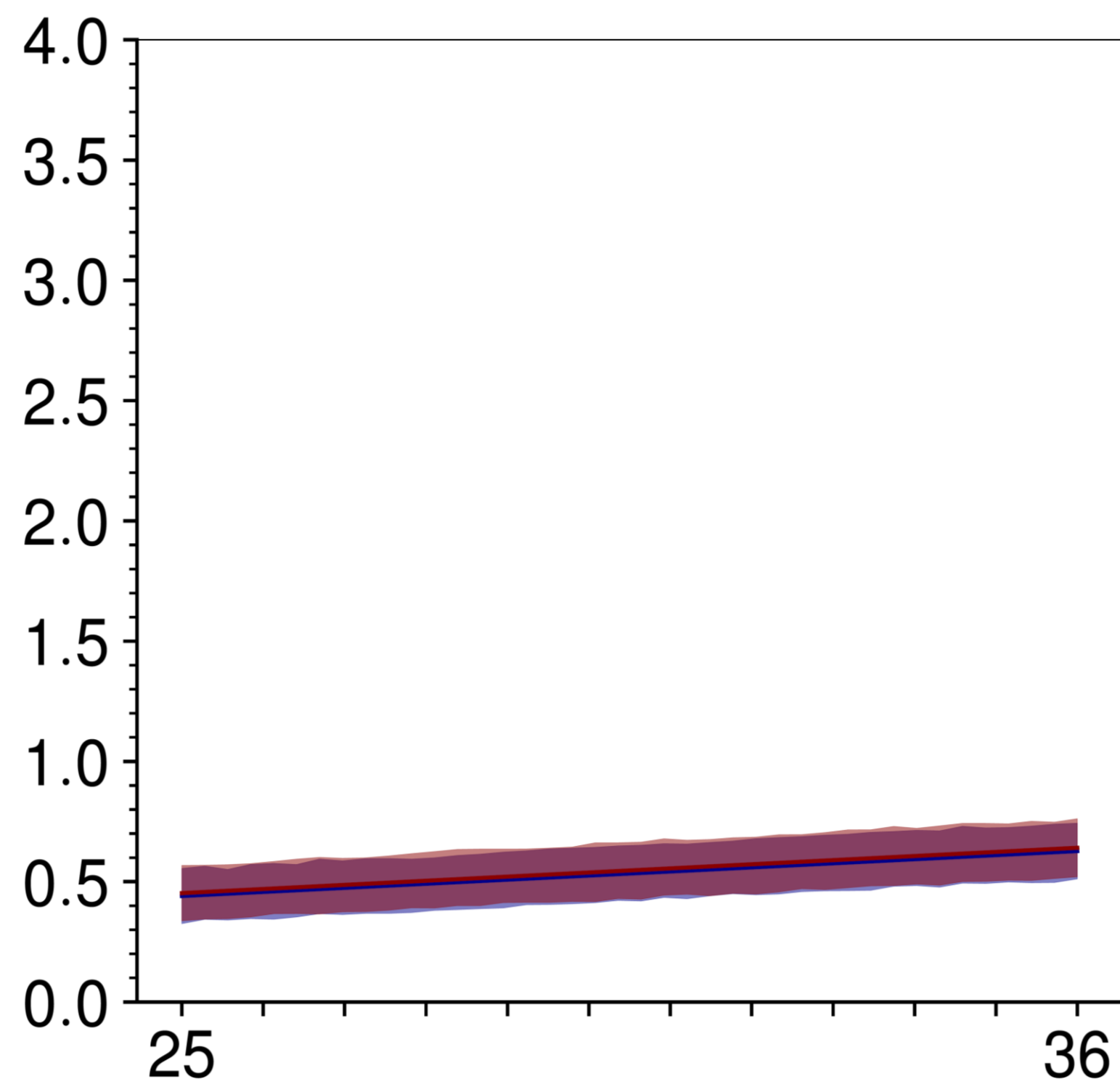
Acclimation (offspring)

Appendix S1

Model description

Life cycle

We simulate a population of maximally N=10000 reproductively mature, diploid individuals with overlapping generations. For the sake of simplicity, we assume that individuals reproduce as hermaphrodites. Simulations can be changed to accommodate for separate sexes, but unless one assumes sex-specific selection and/or small population sizes, there are few differences between hermaphroditic and gonochoristic populations.

Individuals are born as larvae (indicated by the subscript 'lv'). At birth in generation t and day $\tau = i/\ell$, individuals develop a larval phenotype $z_{\mathrm{lv},t+\tau_0}$ according to equation (1) in the main text. They retain this phenotype throughout their larval life until ecclosion to become adults after $\tau_{\mathrm{ad}}\ell$ days, where $\tau_{\mathrm{ad}}\ell = 22$ for Sonora and $\tau_{\mathrm{ad}}\ell = 23$ for Catalina, in line with empirical data on ecclosion time. (However, simulations which used $\tau_{\mathrm{ad}}\ell = 22$ or $\tau_{\mathrm{ad}}\ell = 23$ for either location result in similar outcomes.) All larvae endure daily survival selection, with the probability of survival in generation t and day $\tau_i\ell$ (where $i > 0$) given by $s_{t+\tau_i}\left(z^*_{\mathrm{lv},t+\tau_i}, b^*_{\mathrm{lv},t+\tau_i}, m^*_{\mathrm{lv},t+\tau_i}\right)$ according to equation (2). Here, * denotes a phenotype of an individual who has experienced survival selection. Larvae which ecclose take up adult breeding positions that have been vacated by the $N_{\mathrm{ad,mort}}$ adults who died during the current day. In case there are more subadults than vacancies we randomly select larvae of age $\tau_{\mathrm{ad}}\ell$ days, until all available adult vacancies have been filled and the number of adults is again N, after which the remaining larvae of age $\tau_{\mathrm{ad}}\ell$ are discarded.

Upon becoming an adult, flies again can modulate their phenotype according to within-generational plasticity and maternal effects, according to equation (3) in the main text. Subsequently, also adults survive according to equation (2) in the main text, with the probability of survival in generation t and day $\tau_i \ell$ (where $\tau_{ad}\ell \leq \tau_i \ell < \tau_{max}\ell$) given $s_{t+\tau_i}\left(z_{ad,t+\tau_i}^*, b_{ad,t+\tau_i}^*, m_{ad,t+\tau_i}^*\right)$ according to equation (2). During each day, pairs of randomly selected surviving adults produce a total of $N/(\tau_{ad}\ell)$ newborn larvae. Surviving adults are discarded after they have attained a maximum lifespan of $\tau_{ad}\ell = 28$ days. The individual-based simulations are coded in C++ and are adapted from the individual-based simulations that check analytical results from Kuijper & Hoyle (2015). The code can be found at https://github.com/bramkuijper/maternal_effects_timeseries.

Reaction norms resulting from the experiment

Evolved values of the mean within-generational reaction norm slopes $\bar{b}_{juv}$ and $\bar{b}_{ad}$ and maternal effects $\bar{m}_{juv}$, $\bar{m}_{ad}$ are then being used to simulate the phenotypes developed during the temperature experiment depicted in Figure 1 of the main text. Because we consider cascading maternal effects (McGlothlin & Galloway 2013), any maternal phenotype becomes dependent on the phenotype of her mother and previous ancestors. However, at the start of the experiment, exact phenotypic data about grandmothers and previous ancestors are lacking, hence we decided to take equilibrium values of the mother's adult phenotype $z_{mother,ad}$ who experiences temperature $\varepsilon_{F_0,treatment}$ in the absence of natural selection. Hence,

$$z_{mother,ad}\left(\varepsilon_{F_0,treatment}\right) = \frac{\bar{a} + \bar{b}_{ad}\varepsilon_{F_0,treatment} + e_{mother}}{1 - \bar{m}_{ad}}, \qquad (1)$$

where all values with an overbar are the previously mentioned average phenotypic traits taken from a single replicate simulation. Next, $\varepsilon_{F_0,treatment}$ is the maternal temperature treatment, which is either

25°C or 36°C (see "material and methods" in the main text). As with the original temperature time series, temperatures are standardized relative to the overall temperature mean $E[T] = 0.1070721$ and variance $\sigma_T^2 = 75.83438$ across both locations. Finally, e_{mother} is the amount of developmental noise, which is sampled from a normal distribution with mean 0 and the same variance σ_e^2 as used in the corresponding individual-based simulation (see Table S3). Next, we then obtained the offspring phenotypes expressed as larvae and adults as

$$z_{offspring,lv} = \bar{a} + \bar{b}_{lv}\varepsilon_{F_1,treatment} + \bar{m}_{lv}z_{mother}\left(\varepsilon_{F_0,treatment}\right) + e_{offspring} \qquad (2)$$

$$z_{offspring,ad} = \bar{a} + \bar{b}_{ad}\varepsilon_{F_1,treatment} + \bar{m}_{ad}z_{mother}\left(\varepsilon_{F_0,treatment}\right) + e_{offspring}, \qquad (3)$$

where $\varepsilon_{F_1,treatment}$ treatment reflects the standardized temperature treatment experienced either in larvae or offspring. Reaction norms in Figure 3 are generated by generating the above maternal and offspring phenotypes 50 times for each temperature for each of the 15 replicate simulations.

Appendix S2

Adaptation to temperature timeseries

We model the long-term adaptation of a large-well mixed population of individuals to climatic regimes that mimic the present climate in Sonora and Catalina respectively. To this end, we obtained daily temperature data for both locations provided by National Centers for Environmental Information, National Oceanic and Atmospheric Administration – NOAA (https://www.ncdc.noaa.gov/cdo-web/datasets#NORMAL_HLY), containing two temperature measurements a day for sites close to Sonora (location Sasabe AZ, 31°29'11.39" N 111°32'18.592" W) and Catalina (through measurements on the nearby Sta Rosa Island CA, 33°58' 40.08" N 120°4'40.08" W). Raw temperature data from the national weather service contained intervals where data was missing for multiple years, so we restricted use of temperature data to time intervals for which continuous temperature measurements were available between 23 March 1992 - 21 November 2018 for Sonora and 23 April 1990 - 21 November 2018 for Catalina. In addition, we followed conventional analyses of time series interpolation by imputing smaller intervals of missing data by using Kalman smoothing, using the *na_kalman()* function of the *imputeTS* package in R (Hyndman and Khandakar 2008).

Temperature data was standardized according to $\frac{t_i - \mu}{\sigma}$, where t_i is an individual measurement and μ and σ are the sample mean and standard deviation across both locations and all timepoints. The current analysis focuses on the evolution of maternal effects and within generational plasticity when adapting to different seasonal trends only. Consequently, we used local polynomial regressions through R's

forecast::stl() function to decompose temperature timeseries, allowing us to keep seasonal temperature trends while ignoring longer-term and irregular components (Figures S2 and S3).

As we are interested in how natural selection shapes within-generational and transgenerational plasticity, the length of the resulting standardized temperature timeseries would be far too short to assess evolution of a, b_i and m_i from scratch, unless genetic variation in each trait would be substantial or selection would be extremely strong. This is because 16 and 18 years of temperature data from Sonora and Catalina respectively would cover only 115-130 generations of *D. mojavensis*. Consequently, we chose to concatenate the temperature time series to span 50,000 generations to allow values of all evolving loci to achieve equilibria (see Supplementary Figure S6 for an example simulation in the Sonoran population).

Appendix S3

population	heat-shock_period	temp_parents	temp_acclimation_larva	repl	initial_larva
Catalina	1.5h	Parents 25°C	25°C	1	30
Catalina	1.5h	Parents 25°C	25°C	2	30
Catalina	1.5h	Parents 25°C	25°C	3	30
Catalina	1.5h	Parents 25°C	25°C	4	30
Catalina	1.5h	Parents 25°C	25°C	5	30
Catalina	1.5h	Parents 25°C	25°C	6	30
Catalina	1.5h	Parents 25°C	25°C	7	30
Catalina	1.5h	Parents 25°C	25°C	8	30
Catalina	1.5h	Parents 25°C	25°C	9	30
Catalina	1.5h	Parents 25°C	25°C	10	30
Catalina	1.5h	Parents 25°C	25°C	11	30
Catalina	1.5h	Parents 25°C	25°C	12	30
Catalina	2h	Parents 25°C	25°C	1	30
Catalina	2h	Parents 25°C	25°C	2	30
Catalina	2h	Parents 25°C	25°C	3	30
Catalina	2h	Parents 25°C	25°C	4	30
Catalina	2h	Parents 25°C	25°C	5	30
Catalina	2h	Parents 25°C	25°C	6	30
Catalina	2h	Parents 25°C	25°C	7	30
Catalina	2h	Parents 25°C	25°C	8	30
Catalina	2h	Parents 25°C	25°C	9	30
Catalina	2h	Parents 25°C	25°C	10	30
Catalina	2h	Parents 25°C	25°C	11	30
Catalina	1.5h	Parents 25°C	36°C	1	30
Catalina	1.5h	Parents 25°C	36°C	2	30
Catalina	1.5h	Parents 25°C	36°C	3	30
Catalina	1.5h	Parents 25°C	36°C	4	30
Catalina	1.5h	Parents 25°C	36°C	5	30
Catalina	1.5h	Parents 25°C	36°C	6	30
Catalina	1.5h	Parents 25°C	36°C	7	30
Catalina	1.5h	Parents 25°C	36°C	8	30
Catalina	1.5h	Parents 25°C	36°C	9	30
Catalina	1.5h	Parents 25°C	36°C	10	30
Catalina	1.5h	Parents 25°C	36°C	11	30
Catalina	2h	Parents 25°C	36°C	1	30
Catalina	2h	Parents 25°C	36°C	2	30
Catalina	2h	Parents 25°C	36°C	3	30
Catalina	2h	Parents 25°C	36°C	4	30
Catalina	2h	Parents 25°C	36°C	5	30
Catalina	2h	Parents 25°C	36°C	6	30
Catalina	2h	Parents 25°C	36°C	7	30
Catalina	2h	Parents 25°C	36°C	8	30
Catalina	2h	Parents 25°C	36°C	9	30
Catalina	2h	Parents 25°C	36°C	10	30
Catalina	2h	Parents 25°C	36°C	11	30
Catalina	2h	Parents 25°C	36°C	12	30
Catalina	1.5h	Parents 36°C	25°C	1	30
Catalina	1.5h	Parents 36°C	25°C	2	30
Catalina	1.5h	Parents 36°C	25°C	3	30

Catalina	1.5h	Parents 36°C	25°C	4	30
Catalina	1.5h	Parents 36°C	25°C	5	30
Catalina	1.5h	Parents 36°C	25°C	6	30
Catalina	1.5h	Parents 36°C	25°C	7	30
Catalina	1.5h	Parents 36°C	25°C	8	30
Catalina	1.5h	Parents 36°C	25°C	9	30
Catalina	1.5h	Parents 36°C	25°C	10	30
Catalina	2h	Parents 36°C	25°C	1	30
Catalina	2h	Parents 36°C	25°C	2	30
Catalina	2h	Parents 36°C	25°C	3	30
Catalina	2h	Parents 36°C	25°C	4	30
Catalina	2h	Parents 36°C	25°C	5	30
Catalina	2h	Parents 36°C	25°C	6	30
Catalina	2h	Parents 36°C	25°C	7	30
Catalina	2h	Parents 36°C	25°C	8	30
Catalina	2h	Parents 36°C	25°C	9	30
Catalina	2h	Parents 36°C	25°C	10	30
Catalina	1.5h	Parents 36°C	36°C	1	30
Catalina	1.5h	Parents 36°C	36°C	2	30
Catalina	1.5h	Parents 36°C	36°C	3	30
Catalina	1.5h	Parents 36°C	36°C	4	30
Catalina	1.5h	Parents 36°C	36°C	5	30
Catalina	1.5h	Parents 36°C	36°C	6	30
Catalina	1.5h	Parents 36°C	36°C	7	30
Catalina	1.5h	Parents 36°C	36°C	8	30
Catalina	1.5h	Parents 36°C	36°C	9	30
Catalina	1.5h	Parents 36°C	36°C	10	30
Catalina	2h	Parents 36°C	36°C	1	30
Catalina	2h	Parents 36°C	36°C	2	30
Catalina	2h	Parents 36°C	36°C	3	30
Catalina	2h	Parents 36°C	36°C	4	30
Catalina	2h	Parents 36°C	36°C	5	30
Catalina	2h	Parents 36°C	36°C	6	30
Catalina	2h	Parents 36°C	36°C	7	30
Catalina	2h	Parents 36°C	36°C	8	30
Catalina	2h	Parents 36°C	36°C	9	30
Catalina	2h	Parents 36°C	36°C	10	30
Sonora	1.5h	Parents 25°C	25°C	1	30
Sonora	1.5h	Parents 25°C	25°C	2	30
Sonora	1.5h	Parents 25°C	25°C	3	30
Sonora	1.5h	Parents 25°C	25°C	4	30
Sonora	1.5h	Parents 25°C	25°C	5	30
Sonora	1.5h	Parents 25°C	25°C	6	30
Sonora	1.5h	Parents 25°C	25°C	7	30
Sonora	1.5h	Parents 25°C	25°C	8	30
Sonora	1.5h	Parents 25°C	25°C	9	30
Sonora	1.5h	Parents 25°C	25°C	10	30
Sonora	1.5h	Parents 25°C	25°C	11	30
Sonora	1.5h	Parents 25°C	25°C	12	30
Sonora	2h	Parents 25°C	25°C	1	30
Sonora	2h	Parents 25°C	25°C	2	30
Sonora	2h	Parents 25°C	25°C	3	30
Sonora	2h	Parents 25°C	25°C	4	30

Sonora	2h	Parents 25°C	25°C	5	30
Sonora	2h	Parents 25°C	25°C	6	30
Sonora	2h	Parents 25°C	25°C	7	30
Sonora	2h	Parents 25°C	25°C	8	30
Sonora	2h	Parents 25°C	25°C	9	30
Sonora	2h	Parents 25°C	25°C	10	30
Sonora	2h	Parents 25°C	25°C	11	30
Sonora	1.5h	Parents 25°C	36°C	1	30
Sonora	1.5h	Parents 25°C	36°C	2	30
Sonora	1.5h	Parents 25°C	36°C	3	30
Sonora	1.5h	Parents 25°C	36°C	4	30
Sonora	1.5h	Parents 25°C	36°C	5	30
Sonora	1.5h	Parents 25°C	36°C	6	30
Sonora	1.5h	Parents 25°C	36°C	7	30
Sonora	1.5h	Parents 25°C	36°C	8	30
Sonora	1.5h	Parents 25°C	36°C	9	30
Sonora	1.5h	Parents 25°C	36°C	10	30
Sonora	1.5h	Parents 25°C	36°C	11	30
Sonora	1.5h	Parents 25°C	36°C	12	30
Sonora	2h	Parents 25°C	36°C	1	30
Sonora	2h	Parents 25°C	36°C	2	30
Sonora	2h	Parents 25°C	36°C	3	30
Sonora	2h	Parents 25°C	36°C	4	30
Sonora	2h	Parents 25°C	36°C	5	30
Sonora	2h	Parents 25°C	36°C	6	30
Sonora	2h	Parents 25°C	36°C	7	30
Sonora	2h	Parents 25°C	36°C	8	30
Sonora	2h	Parents 25°C	36°C	9	30
Sonora	2h	Parents 25°C	36°C	10	30
Sonora	2h	Parents 25°C	36°C	11	30
Sonora	2h	Parents 25°C	36°C	12	30
Sonora	1.5h	Parents 36°C	25°C	1	30
Sonora	1.5h	Parents 36°C	25°C	2	30
Sonora	1.5h	Parents 36°C	25°C	3	30
Sonora	1.5h	Parents 36°C	25°C	4	30
Sonora	1.5h	Parents 36°C	25°C	5	30
Sonora	1.5h	Parents 36°C	25°C	6	30
Sonora	1.5h	Parents 36°C	25°C	7	30
Sonora	1.5h	Parents 36°C	25°C	8	30
Sonora	1.5h	Parents 36°C	25°C	9	30
Sonora	1.5h	Parents 36°C	25°C	10	30
Sonora	1.5h	Parents 36°C	25°C	11	30
Sonora	1.5h	Parents 36°C	25°C	12	30
Sonora	2h	Parents 36°C	25°C	1	30
Sonora	2h	Parents 36°C	25°C	2	30
Sonora	2h	Parents 36°C	25°C	3	30
Sonora	2h	Parents 36°C	25°C	4	30
Sonora	2h	Parents 36°C	25°C	5	30
Sonora	2h	Parents 36°C	25°C	6	30
Sonora	2h	Parents 36°C	25°C	7	30
Sonora	2h	Parents 36°C	25°C	8	30
Sonora	2h	Parents 36°C	25°C	9	30
Sonora	2h	Parents 36°C	25°C	10	30

Sonora	2h	Parents 36°C	25°C	11	30
Sonora	2h	Parents 36°C	25°C	12	30
Sonora	1.5h	Parents 36°C	36°C	1	30
Sonora	1.5h	Parents 36°C	36°C	2	30
Sonora	1.5h	Parents 36°C	36°C	3	30
Sonora	1.5h	Parents 36°C	36°C	4	30
Sonora	1.5h	Parents 36°C	36°C	5	30
Sonora	1.5h	Parents 36°C	36°C	6	30
Sonora	1.5h	Parents 36°C	36°C	7	30
Sonora	1.5h	Parents 36°C	36°C	8	30
Sonora	1.5h	Parents 36°C	36°C	9	30
Sonora	1.5h	Parents 36°C	36°C	10	30
Sonora	1.5h	Parents 36°C	36°C	11	30
Sonora	1.5h	Parents 36°C	36°C	12	30
Sonora	2h	Parents 36°C	36°C	1	30
Sonora	2h	Parents 36°C	36°C	2	30
Sonora	2h	Parents 36°C	36°C	3	30
Sonora	2h	Parents 36°C	36°C	4	30
Sonora	2h	Parents 36°C	36°C	5	30
Sonora	2h	Parents 36°C	36°C	6	30
Sonora	2h	Parents 36°C	36°C	7	30
Sonora	2h	Parents 36°C	36°C	8	30
Sonora	2h	Parents 36°C	36°C	9	30
Sonora	2h	Parents 36°C	36°C	10	30
Sonora	2h	Parents 36°C	36°C	11	30
Sonora	2h	Parents 36°C	36°C	12	30

num_pupa	num_adults	larva-pupa_viability	larva-adult_viability	larva-pupa_std_viability
15	15	0.5	0.5	0.547
2	2	0.067	0.067	0.073
21	21	0.7	0.7	0.766
13	12	0.433	0.4	0.474
17	17	0.567	0.567	0.62
21	21	0.7	0.7	0.766
5	3	0.167	0.1	0.182
18	18	0.6	0.6	0.657
19	18	0.633	0.6	0.693
23	22	0.767	0.733	0.839
22	22	0.733	0.733	0.803
23	21	0.767	0.7	0.839
8	8	0.267	0.267	0.292
1	1	0.033	0.033	0.036
18	18	0.6	0.6	0.657
12	11	0.4	0.367	0.438
8	8	0.267	0.267	0.292
0	0	0	0	0
0	0	0	0	0
10	10	0.333	0.333	0.365
6	4	0.2	0.133	0.219
8	8	0.267	0.267	0.292
20	19	0.667	0.633	0.73
13	12	0.433	0.4	0.628
15	15	0.5	0.5	0.725
23	23	0.767	0.767	1.111
20	20	0.667	0.667	0.966
19	19	0.633	0.633	0.918
1	1	0.033	0.033	0.048
4	4	0.133	0.133	0.193
17	17	0.567	0.567	0.821
23	21	0.767	0.7	1.111
29	29	0.967	0.967	1.401
21	21	0.7	0.7	1.014
12	10	0.4	0.333	0.58
16	16	0.533	0.533	0.773
20	19	0.667	0.633	0.966
26	27	0.867	0.9	1.256
7	7	0.233	0.233	0.338
8	8	0.267	0.267	0.386
9	8	0.3	0.267	0.435
11	11	0.367	0.367	0.531
25	22	0.833	0.733	1.208
18	16	0.6	0.533	0.87
18	15	0.6	0.5	0.87
20	20	0.667	0.667	0.966
26	26	0.867	0.867	0.974
26	25	0.867	0.833	0.974
27	25	0.9	0.833	1.011

27	27	0.9	0.9	1.011
16	10	0.533	0.333	0.599
16	15	0.533	0.5	0.599
20	20	0.667	0.667	0.749
22	22	0.733	0.733	0.824
29	29	0.967	0.967	1.086
21	21	0.7	0.7	0.787
21	21	0.7	0.7	0.787
20	20	0.667	0.667	0.749
22	21	0.733	0.7	0.824
10	9	0.333	0.3	0.375
6	6	0.2	0.2	0.225
7	7	0.233	0.233	0.262
14	14	0.467	0.467	0.524
22	22	0.733	0.733	0.824
23	22	0.767	0.733	0.861
24	23	0.8	0.767	0.899
23	22	0.767	0.733	1.205
13	13	0.433	0.433	0.681
18	17	0.6	0.567	0.943
21	19	0.7	0.633	1.1
20	20	0.667	0.667	1.048
14	14	0.467	0.467	0.733
18	18	0.6	0.6	0.943
23	23	0.767	0.767	1.205
26	25	0.867	0.833	1.362
21	20	0.7	0.667	1.1
16	16	0.533	0.533	0.838
15	15	0.5	0.5	0.786
21	20	0.7	0.667	1.1
16	16	0.533	0.533	0.838
24	22	0.8	0.733	1.257
18	17	0.6	0.567	0.943
12	12	0.4	0.4	0.629
0	0	0	0	0
24	21	0.8	0.7	1.257
20	18	0.667	0.6	1.048
5	5	0.167	0.167	0.179
6	6	0.2	0.2	0.215
5	5	0.167	0.167	0.179
8	8	0.267	0.267	0.287
5	5	0.167	0.167	0.179
9	8	0.3	0.267	0.323
4	4	0.133	0.133	0.143
11	11	0.367	0.367	0.394
4	4	0.133	0.133	0.143
9	9	0.3	0.3	0.323
na	na	na	na	na
na	na	na	na	na
0	0	0	0	0
2	2	0.067	0.067	0.072
3	3	0.1	0.1	0.108
5	5	0.167	0.167	0.179

1	1	0.033	0.033	0.036
4	4	0.133	0.133	0.143
2	2	0.067	0.067	0.072
2	2	0.067	0.067	0.072
3	3	0.1	0.1	0.108
0	0	0	0	0
7	7	0.233	0.233	0.251
10	10	0.333	0.333	0.641
16	15	0.533	0.5	1.026
10	10	0.333	0.333	0.641
10	10	0.333	0.333	0.641
7	6	0.233	0.2	0.449
0	0	0	0	0
12	12	0.4	0.4	0.769
10	9	0.333	0.3	0.641
0	0	0	0	0
17	16	0.567	0.533	1.09
na	na	na	na	na
na	na	na	na	na
13	13	0.433	0.433	0.833
13	13	0.433	0.433	0.833
4	4	0.133	0.133	0.256
15	15	0.5	0.5	0.962
0	0	0	0	0
3	3	0.1	0.1	0.192
7	7	0.233	0.233	0.449
0	0	0	0	0
10	10	0.333	0.333	0.641
0	0	0	0	0
na	na	na	na	na
na	na	na	na	na
13	11	0.433	0.367	0.518
12	12	0.4	0.4	0.478
10	10	0.333	0.333	0.398
15	13	0.5	0.433	0.598
18	16	0.6	0.533	0.717
10	10	0.333	0.333	0.398
0	0	0	0	0
6	6	0.2	0.2	0.239
13	13	0.433	0.433	0.518
7	7	0.233	0.233	0.279
na	na	na	na	na
na	na	na	na	na
10	9	0.333	0.3	0.398
7	7	0.233	0.233	0.279
5	5	0.167	0.167	0.199
5	5	0.167	0.167	0.199
5	5	0.167	0.167	0.199
6	6	0.2	0.2	0.239
0	0	0	0	0
4	3	0.133	0.1	0.159
11	11	0.367	0.367	0.438
8	8	0.267	0.267	0.319

na	na	na	na	na
na	na	na	na	na
15	15	0.5	0.5	1.103
17	17	0.567	0.567	1.25
12	12	0.4	0.4	0.882
18	18	0.6	0.6	1.324
18	18	0.6	0.6	1.324
12	12	0.4	0.4	0.882
10	10	0.333	0.333	0.735
0	0	0	0	0
12	10	0.4	0.333	0.882
9	9	0.3	0.3	0.662
na	na	na	na	na
4	4	0.133	0.133	0.294
12	11	0.4	0.367	0.882
17	14	0.567	0.467	1.25
17	17	0.567	0.567	1.25
11	11	0.367	0.367	0.809
12	12	0.4	0.4	0.882
12	11	0.4	0.367	0.882
8	7	0.267	0.233	0.588
13	13	0.433	0.433	0.956
18	18	0.6	0.6	1.324
3	3	0.1	0.1	0.221
9	9	0.3	0.3	0.662
15	15	0.5	0.5	1.103

larva-adult_std_viability
0.564
0.075
0.789
0.451
0.639
0.789
0.113
0.677
0.677
0.827
0.827
0.789
0.301
0.038
0.677
0.414
0.301
0
0
0.376
0.15
0.301
0.714
0.588
0.735
1.127
0.98
0.931
0.049
0.196
0.833
1.029
1.422
1.029
0.49
0.784
0.931
1.324
0.343
0.392
0.392
0.539
1.078
0.784
0.735
0.98
0.996
0.958
0.958

1.034
0.383
0.575
0.766
0.843
1.111
0.805
0.805
0.766
0.805
0.345
0.23
0.268
0.536
0.843
0.843
0.881
1.204
0.711
0.93
1.04
1.095
0.766
0.985
1.259
1.368
1.095
0.876
0.821
1.095
0.876
1.204
0.93
0.657
0
1.149
0.985
0.195
0.233
0.195
0.311
0.195
0.311
0.156
0.428
0.156
0.35
na
na
0
0.078
0.117
0.195

0.039
0.156
0.078
0.078
0.117
0
0.272
0.667
1
0.667
0.667
0.4
0
0.8
0.6
0
1.067
na
na
0.867
0.867
0.267
1
0
0.2
0.467
0
0.667
0
na
na
0.478
0.522
0.435
0.565
0.696
0.435
0
0.261
0.565
0.304
na
na
0.391
0.304
0.217
0.217
0.217
0.261
0
0.13
0.478
0.348

na
na
1.128
1.278
0.902
1.353
1.353
0.902
0.752
0
0.752
0.677
na
0.301
0.827
1.053
1.278
0.827
0.902
0.827
0.526
0.977
1.353
0.226
0.677
1.128

Appendix S3

population	heat-shock_period	temp_parents	temp_acclimation_larva	repl	dev_pupa	dev_adults
Catalina	1.5h	Parents 25 °C	25°C	1	na	na
Catalina	1.5h	Parents 25 °C	25°C	2	na	na
Catalina	1.5h	Parents 25 °C	25°C	3	10	15.76
Catalina	1.5h	Parents 25 °C	25°C	4	10.08	16.17
Catalina	1.5h	Parents 25 °C	25°C	5	10.06	15.71
Catalina	1.5h	Parents 25 °C	25°C	6	10.62	16.24
Catalina	1.5h	Parents 25 °C	25°C	7	na	na
Catalina	1.5h	Parents 25 °C	25°C	8	11.56	16.56
Catalina	1.5h	Parents 25 °C	25°C	9	11	15.78
Catalina	1.5h	Parents 25 °C	25°C	10	10.52	15.27
Catalina	1.5h	Parents 25 °C	25°C	11	9.59	15.36
Catalina	1.5h	Parents 25 °C	25°C	12	9.83	14.9
Catalina	2h	Parents 25 °C	25°C	1	na	na
Catalina	2h	Parents 25 °C	25°C	2	na	na
Catalina	2h	Parents 25 °C	25°C	3	9.33	14.78
Catalina	2h	Parents 25 °C	25°C	4	9.25	15
Catalina	2h	Parents 25 °C	25°C	5	13.5	19.13
Catalina	2h	Parents 25 °C	25°C	6	na	na
Catalina	2h	Parents 25 °C	25°C	7	na	na
Catalina	2h	Parents 25 °C	25°C	8	11.67	16.5
Catalina	2h	Parents 25 °C	25°C	9	11.83	16.5
Catalina	2h	Parents 25 °C	25°C	10	10.75	16
Catalina	2h	Parents 25 °C	25°C	11	9.95	15.53
Catalina	1.5h	Parents 25 °C	36°C	1	na	na
Catalina	1.5h	Parents 25 °C	36°C	2	na	na
Catalina	1.5h	Parents 25 °C	36°C	3	10.7	16.74
Catalina	1.5h	Parents 25 °C	36°C	4	9.95	14.7
Catalina	1.5h	Parents 25 °C	36°C	5	12.46	17.26
Catalina	1.5h	Parents 25 °C	36°C	6	na	na
Catalina	1.5h	Parents 25 °C	36°C	7	12.67	20.25
Catalina	1.5h	Parents 25 °C	36°C	8	11.59	16.41
Catalina	1.5h	Parents 25 °C	36°C	9	10.87	15.81
Catalina	1.5h	Parents 25 °C	36°C	10	11.31	16.24
Catalina	1.5h	Parents 25 °C	36°C	11	11.89	17.9
Catalina	2h	Parents 25 °C	36°C	1	na	na
Catalina	2h	Parents 25 °C	36°C	2	na	na
Catalina	2h	Parents 25 °C	36°C	3	9.45	15.26
Catalina	2h	Parents 25 °C	36°C	4	10.19	16.19
Catalina	2h	Parents 25 °C	36°C	5	13	18.57
Catalina	2h	Parents 25 °C	36°C	6	15.14	21
Catalina	2h	Parents 25 °C	36°C	7	13.11	19
Catalina	2h	Parents 25 °C	36°C	8	16	19.82
Catalina	2h	Parents 25 °C	36°C	9	11.12	16.82
Catalina	2h	Parents 25 °C	36°C	10	10.22	15.75
Catalina	2h	Parents 25 °C	36°C	11	11.78	17.4
Catalina	2h	Parents 25 °C	36°C	12	12.65	18.2
Catalina	1.5h	Parents 36 °C	25°C	1	na	na
Catalina	1.5h	Parents 36 °C	25°C	2	na	na
Catalina	1.5h	Parents 36 °C	25°C	3	9.41	15.08

Catalina	1.5h	Parents 36 °C	25°C	4	12.59	15.26
Catalina	1.5h	Parents 36 °C	25°C	5	10.44	16.8
Catalina	1.5h	Parents 36 °C	25°C	6	10.63	16.27
Catalina	1.5h	Parents 36 °C	25°C	7	11.05	16.1
Catalina	1.5h	Parents 36 °C	25°C	8	11.09	16.41
Catalina	1.5h	Parents 36 °C	25°C	9	10.66	15.93
Catalina	1.5h	Parents 36 °C	25°C	10	10	15.52
Catalina	2h	Parents 36 °C	25°C	1	na	na
Catalina	2h	Parents 36 °C	25°C	2	na	na
Catalina	2h	Parents 36 °C	25°C	3	8.95	14.81
Catalina	2h	Parents 36 °C	25°C	4	7.6	13.11
Catalina	2h	Parents 36 °C	25°C	5	12	17
Catalina	2h	Parents 36 °C	25°C	6	10.71	16.71
Catalina	2h	Parents 36 °C	25°C	7	12.07	17.5
Catalina	2h	Parents 36 °C	25°C	8	10	15.32
Catalina	2h	Parents 36 °C	25°C	9	10.35	15.73
Catalina	2h	Parents 36 °C	25°C	10	10.42	15.83
Catalina	1.5h	Parents 36 °C	36°C	1	na	na
Catalina	1.5h	Parents 36 °C	36°C	2	na	na
Catalina	1.5h	Parents 36 °C	36°C	3	8.5	14.18
Catalina	1.5h	Parents 36 °C	36°C	4	9.33	14.63
Catalina	1.5h	Parents 36 °C	36°C	5	10.1	15.85
Catalina	1.5h	Parents 36 °C	36°C	6	9.86	15.57
Catalina	1.5h	Parents 36 °C	36°C	7	10.5	16.11
Catalina	1.5h	Parents 36 °C	36°C	8	10.87	16.22
Catalina	1.5h	Parents 36 °C	36°C	9	10.15	15.76
Catalina	1.5h	Parents 36 °C	36°C	10	10.48	15.65
Catalina	2h	Parents 36 °C	36°C	1	na	na
Catalina	2h	Parents 36 °C	36°C	2	na	na
Catalina	2h	Parents 36 °C	36°C	3	9.86	15.3
Catalina	2h	Parents 36 °C	36°C	4	12.31	18
Catalina	2h	Parents 36 °C	36°C	5	9.71	15.5
Catalina	2h	Parents 36 °C	36°C	6	9.56	15.24
Catalina	2h	Parents 36 °C	36°C	7	11	16.42
Catalina	2h	Parents 36 °C	36°C	8	na	na
Catalina	2h	Parents 36 °C	36°C	9	10.08	15.62
Catalina	2h	Parents 36 °C	36°C	10	9.95	15.11
Sonora	1.5h	Parents 25 °C	25°C	1	na	na
Sonora	1.5h	Parents 25 °C	25°C	2	na	na
Sonora	1.5h	Parents 25 °C	25°C	3	10	15.8
Sonora	1.5h	Parents 25 °C	25°C	4	11.5	17
Sonora	1.5h	Parents 25 °C	25°C	5	14	19.4
Sonora	1.5h	Parents 25 °C	25°C	6	11.22	17.25
Sonora	1.5h	Parents 25 °C	25°C	7	13.25	18.25
Sonora	1.5h	Parents 25 °C	25°C	8	11.18	16.45
Sonora	1.5h	Parents 25 °C	25°C	9	13.75	18.33
Sonora	1.5h	Parents 25 °C	25°C	10	11	16.33
Sonora	1.5h	Parents 25 °C	25°C	11	na	na
Sonora	1.5h	Parents 25 °C	25°C	12	na	na
Sonora	2h	Parents 25 °C	25°C	1	na	na
Sonora	2h	Parents 25 °C	25°C	2	na	na
Sonora	2h	Parents 25 °C	25°C	3	10.33	15.67
Sonora	2h	Parents 25 °C	25°C	4	11.4	17

Sonora	2h	Parents 25 °C	25°C	5	10	16
Sonora	2h	Parents 25 °C	25°C	6	12	17.5
Sonora	2h	Parents 25 °C	25°C	7	12	17
Sonora	2h	Parents 25 °C	25°C	8	12.5	17.5
Sonora	2h	Parents 25 °C	25°C	9	12.33	17.33
Sonora	2h	Parents 25 °C	25°C	10	na	na
Sonora	2h	Parents 25 °C	25°C	11	10.43	15.57
Sonora	1.5h	Parents 25 °C	36°C	1	na	na
Sonora	1.5h	Parents 25 °C	36°C	2	na	na
Sonora	1.5h	Parents 25 °C	36°C	3	10.3	16
Sonora	1.5h	Parents 25 °C	36°C	4	10.6	16.3
Sonora	1.5h	Parents 25 °C	36°C	5	12.71	18.5
Sonora	1.5h	Parents 25 °C	36°C	6	na	na
Sonora	1.5h	Parents 25 °C	36°C	7	11.67	16.92
Sonora	1.5h	Parents 25 °C	36°C	8	13	18.22
Sonora	1.5h	Parents 25 °C	36°C	9	na	na
Sonora	1.5h	Parents 25 °C	36°C	10	12.29	17.31
Sonora	1.5h	Parents 25 °C	36°C	11	na	na
Sonora	1.5h	Parents 25 °C	36°C	12	na	na
Sonora	2h	Parents 25 °C	36°C	1	na	na
Sonora	2h	Parents 25 °C	36°C	2	na	na
Sonora	2h	Parents 25 °C	36°C	3	11	17
Sonora	2h	Parents 25 °C	36°C	4	11.2	16.47
Sonora	2h	Parents 25 °C	36°C	5	na	na
Sonora	2h	Parents 25 °C	36°C	6	13.67	19
Sonora	2h	Parents 25 °C	36°C	7	12	17.29
Sonora	2h	Parents 25 °C	36°C	8	na	na
Sonora	2h	Parents 25 °C	36°C	9	13.3	18.8
Sonora	2h	Parents 25 °C	36°C	10	na	na
Sonora	2h	Parents 25 °C	36°C	11	na	na
Sonora	2h	Parents 25 °C	36°C	12	na	na
Sonora	1.5h	Parents 36 °C	25°C	1	na	na
Sonora	1.5h	Parents 36 °C	25°C	2	na	na
Sonora	1.5h	Parents 36 °C	25°C	3	10.5	16.3
Sonora	1.5h	Parents 36 °C	25°C	4	10.87	16.54
Sonora	1.5h	Parents 36 °C	25°C	5	11.78	17.31
Sonora	1.5h	Parents 36 °C	25°C	6	10.6	16.1
Sonora	1.5h	Parents 36 °C	25°C	7	na	na
Sonora	1.5h	Parents 36 °C	25°C	8	13	18.17
Sonora	1.5h	Parents 36 °C	25°C	9	10.23	15.69
Sonora	1.5h	Parents 36 °C	25°C	10	11.43	16.57
Sonora	1.5h	Parents 36 °C	25°C	11	na	na
Sonora	1.5h	Parents 36 °C	25°C	12	na	na
Sonora	2h	Parents 36 °C	25°C	1	na	na
Sonora	2h	Parents 36 °C	25°C	2	na	na
Sonora	2h	Parents 36 °C	25°C	3	10.4	16
Sonora	2h	Parents 36 °C	25°C	4	10.6	15.4
Sonora	2h	Parents 36 °C	25°C	5	11.2	17.2
Sonora	2h	Parents 36 °C	25°C	6	11	16.67
Sonora	2h	Parents 36 °C	25°C	7	na	na
Sonora	2h	Parents 36 °C	25°C	8	15	20.33
Sonora	2h	Parents 36 °C	25°C	9	10.55	16
Sonora	2h	Parents 36 °C	25°C	10	11.86	17.25

Sonora	2h	Parents 36 °C	25°C	11	na	na
Sonora	2h	Parents 36 °C	25°C	12	na	na
Sonora	1.5h	Parents 36 °C	36°C	1	na	na
Sonora	1.5h	Parents 36 °C	36°C	2	na	na
Sonora	1.5h	Parents 36 °C	36°C	3	14.25	19.83
Sonora	1.5h	Parents 36 °C	36°C	4	11.59	16.94
Sonora	1.5h	Parents 36 °C	36°C	5	12.19	18.5
Sonora	1.5h	Parents 36 °C	36°C	6	14.27	20.58
Sonora	1.5h	Parents 36 °C	36°C	7	13.5	18.9
Sonora	1.5h	Parents 36 °C	36°C	8	na	na
Sonora	1.5h	Parents 36 °C	36°C	9	12.5	17.6
Sonora	1.5h	Parents 36 °C	36°C	10	14.43	20.67
Sonora	1.5h	Parents 36 °C	36°C	11	na	na
Sonora	1.5h	Parents 36 °C	36°C	12	14	23
Sonora	2h	Parents 36 °C	36°C	1	na	na
Sonora	2h	Parents 36 °C	36°C	2	na	na
Sonora	2h	Parents 36 °C	36°C	3	11.65	17.35
Sonora	2h	Parents 36 °C	36°C	4	13.64	19.64
Sonora	2h	Parents 36 °C	36°C	5	15.25	21.17
Sonora	2h	Parents 36 °C	36°C	6	16.08	22.36
Sonora	2h	Parents 36 °C	36°C	7	16.75	21.71
Sonora	2h	Parents 36 °C	36°C	8	11.77	17.15
Sonora	2h	Parents 36 °C	36°C	9	12.59	17.89
Sonora	2h	Parents 36 °C	36°C	10	15.33	20.67
Sonora	2h	Parents 36 °C	36°C	11	12.78	17.89
Sonora	2h	Parents 36 °C	36°C	12	10.4	16.47

dev_pupa_adults

na
na
5.76
6.09
5.65
5.62
na
5
4.78
4.75
5.77
5.08
na
na
5.44
5.75
5.63
na
na
4.83
4.67
5.25
5.58
na
na
6.04
4.75
4.8
na
7.58
4.82
4.94
4.93
6.02
na
na
5.81
5.99
5.57
5.86
5.89
3.82
5.7
5.53
5.62
5.55
na
na
5.67

2.67
6.36
5.64
5.05
5.32
5.28
5.52
na
na
5.85
5.51
5
6
5.43
5.32
5.38
5.41
na
na
5.68
5.3
5.75
5.71
5.61
5.35
5.61
5.17
na
na
5.44
5.69
5.79
5.68
5.42
na
5.54
5.16
na
na
5.8
5.5
5.4
6.03
5
5.27
4.58
5.33
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6
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5.67
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5.5
na
5.17
5.46
5.14
na
na
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na
5.6
4.8
6
5.67
na
5.33
5.45
5.39

na
na
na
na
5.58
5.36
6.31
6.31
5.4
na
5.1
6.24
na
9
na
na
5.71
6
5.92
6.28
4.96
5.38
5.3
5.33
5.11
6.07

Appendix S3

population	temp_parents	temp_acclimation_larva	temp_acclimation_adult	sex	repl	hrs	min
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	1	2	23
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	2	2	23
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	3	2	27
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	4	2	36
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	5	2	24
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	6	2	47
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	7	2	44
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	8	2	53
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	9	2	28
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	10	2	37
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	11	2	37
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	12	2	39
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	13	2	25
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	14	2	56
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	15	3	0
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	16	2	52
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	17	3	11
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	18	3	8
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	19	1	53
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	20	2	48
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	21	3	3
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	22	2	46
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	23	2	20
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	24	2	32
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	25	2	56
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	26	2	42
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	27	3	10
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	28	2	21
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	29	3	12
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	30	2	29
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	31	2	40
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	32	2	30
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	33	2	51
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	34	2	32
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	35	2	43
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	36	3	18
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	37	2	47
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	38	2	33
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	39	3	30
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	40	1	55
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	41	1	48
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	42	1	54
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	43	1	56
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	44	2	17
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	45	2	50
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	46	1	56
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	47	1	56
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	48	2	58
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	25	2	41

Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	26	2	20
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	27	2	37
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	28	2	45
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	29	3	14
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	30	2	51
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	31	2	21
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	32	2	8
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	33	2	21
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	34	2	46
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	35	2	8
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	36	2	28
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	37	2	47
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	38	3	21
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	39	2	51
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	40	2	42
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	41	3	20
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	42	3	5
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	43	2	34
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	44	3	30
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	45	3	2
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	46	2	49
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	47	2	39
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	48	3	5
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	1	2	44
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	2	3	6
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	3	2	53
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	4	2	35
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	5	2	49
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	6	3	3
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	7	2	15
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	8	3	13
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	9	2	6
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	10	2	16
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	11	2	35
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	12	1	52
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	13	2	31
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	14	2	13
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	15	2	4
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	16	2	50
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	17	2	45
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	18	2	27
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	19	2	36
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	20	2	59
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	21	3	15
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	22	2	45
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	23	3	30
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	24	3	18
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	24	2	48
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	23	3	0
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	22	2	34
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	21	2	48
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	20	3	17
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	19	3	10

Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	18	2	55
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	17	3	5
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	16	2	13
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	15	2	54
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	14	2	53
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	13	2	42
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	12	2	42
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	11	3	27
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	10	3	19
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	9	2	47
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	8	3	30
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	7	3	13
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	6	2	41
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	5	2	59
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	4	3	8
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	3	3	18
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	2	3	30
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	1	3	8
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	48	2	14
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	47	3	7
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	46	3	7
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	45	2	34
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	44	2	58
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	43	2	58
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	42	2	58
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	41	3	12
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	40	3	15
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	39	2	58
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	38	2	45
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	37	3	12
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	36	3	0
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	35	3	11
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	34	3	24
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	33	2	56
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	32	3	30
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	31	3	0
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	30	3	30
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	29	3	0
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	28	3	30
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	27	3	11
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	26	3	24
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	25	3	30
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	7	2	16
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	8	2	26
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	9	2	59
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	10	2	21
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	11	2	43
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	12	2	32
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	1	2	56
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	2	2	47
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	3	2	31
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	4	2	36
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	5	2	42

Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	6	2	26
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	19	2	37
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	20	2	47
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	21	3	17
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	22	2	44
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	23	2	52
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	24	3	0
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	13	2	28
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	14	3	25
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	15	2	47
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	16	2	28
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	17	3	5
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	18	3	11
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	31	2	58
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	32	2	34
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	33	3	12
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	34	2	53
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	35	2	49
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	36	2	30
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	25	2	33
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	26	2	44
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	27	2	39
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	28	2	18
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	29	3	14
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	30	2	57
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	43	3	13
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	44	3	1
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	45	2	54
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	46	3	1
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	47	3	8
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	48	3	30
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	37	2	24
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	38	3	1
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	39	3	30
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	40	2	46
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	41	2	54
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	42	2	41
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	31	2	26
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	32	3	3
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	33	3	3
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	34	2	41
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	35	2	13
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	36	3	7
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	25	2	46
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	26	3	0
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	27	2	39
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	28	2	29
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	29	3	0
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	30	2	47
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	43	3	14
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	44	3	30
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	45	3	2
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	46	2	42

Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	47	3	30
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	48	3	26
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	37	2	53
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	38	3	13
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	39	3	30
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	40	2	17
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	41	2	54
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	42	3	10
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	7	2	44
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	8	2	32
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	9	2	56
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	10	2	32
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	11	2	57
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	12	2	57
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	1	2	20
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	2	2	20
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	3	2	21
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	4	2	43
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	5	2	55
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	6	2	32
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	19	2	59
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	20	3	30
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	21	3	12
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	22	2	11
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	23	3	0
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	24	3	0
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	13	2	5
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	14	2	37
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	15	3	19
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	16	2	58
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	17	2	45
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	18	3	30
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	19	2	32
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	20	2	51
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	21	3	1
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	22	3	1
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	23	3	2
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	24	3	2
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	13	2	48
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	14	2	48
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	15	3	0
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	16	2	25
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	17	3	1
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	18	2	39
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	7	2	46
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	8	2	30
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	9	3	19
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	10	3	22
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	11	2	58
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	12	2	48
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	1	3	8
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	2	3	27
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	3	3	30

Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	4	2	45
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	5	2	58
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	6	3	5
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	43	2	43
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	44	2	36
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	45	3	7
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	46	2	56
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	47	2	56
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	48	2	56
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	37	2	54
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	38	2	35
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	39	2	55
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	40	2	55
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	41	2	6
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	42	3	3
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	31	3	12
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	32	2	52
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	33	3	30
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	34	2	53
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	35	2	53
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	36	3	30
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	25	2	52
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	26	2	34
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	27	3	2
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	28	2	41
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	29	3	6
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	30	3	30
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	48	2	28
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	47	3	2
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	46	3	2
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	45	2	41
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	44	2	10
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	43	2	41
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	42	2	17
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	41	2	40
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	40	2	47
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	39	2	47
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	38	2	53
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	37	2	53
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	36	2	52
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	35	3	6
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	34	2	57
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	33	2	39
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	32	3	1
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	31	3	5
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	30	2	26
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	29	3	30
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	28	3	11
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	27	2	56
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	26	2	51
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	25	3	17
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	24	2	14
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	23	2	25

Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	22	2	50
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	21	2	9
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	20	2	32
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	19	2	55
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	18	2	24
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	17	2	50
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	16	2	12
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	15	2	23
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	14	3	0
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	13	2	50
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	12	2	30
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	11	3	20
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	10	2	59
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	9	2	43
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	8	3	30
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	7	3	30
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	6	2	22
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	5	2	49
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	4	1	27
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	3	2	48
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	2	1	31
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	1	3	3
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	7	2	54
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	8	2	48
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	9	2	48
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	10	2	58
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	11	2	28
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	12	2	55
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	1	2	20
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	2	2	10
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	3	3	16
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	4	2	54
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	5	2	58
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	6	3	12
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	19	2	55
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	20	3	12
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	21	3	12
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	22	3	10
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	23	2	43
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	24	3	7
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	13	2	49
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	14	2	41
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	15	2	49
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	16	2	42
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	17	2	50
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	18	3	30
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	31	2	31
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	32	2	44
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	33	2	31
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	34	2	37
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	35	2	25
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	36	2	37
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	25	2	56

Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	26	2	23
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	27	2	52
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	28	2	44
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	29	2	31
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	30	2	57
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	43	3	12
Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	44	2	38
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	45	3	9
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	46	2	46
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	47	2	53
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	48	2	33
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	37	3	8
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	38	3	12
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	39	2	57
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	40	2	38
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	41	2	38
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	42	3	12
Sonora	Parents 25 °C	Larva 25 °C	25 °C	M	37	2	17
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	38	2	28
Sonora	Parents 25 °C	Larva 25 °C	25 °C	F	39	2	56
Sonora	Parents 25 °C	Larva 36 °C	25 °C	M	40	3	1
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	41	2	40
Sonora	Parents 25 °C	Larva 36 °C	25 °C	F	42	2	52
Sonora	Parents 36 °C	Larva 25 °C	25 °C	M	43	2	9
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	44	2	52
Sonora	Parents 36 °C	Larva 25 °C	25 °C	F	45	2	52
Sonora	Parents 36 °C	Larva 36 °C	25 °C	M	46	2	36
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	47	2	40
Sonora	Parents 36 °C	Larva 36 °C	25 °C	F	48	3	25
Sonora	Parents 25 °C	Larva 25 °C	36 °C	M	25	2	26
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	26	2	55
Sonora	Parents 25 °C	Larva 25 °C	36 °C	F	27	3	4
Sonora	Parents 25 °C	Larva 36 °C	36 °C	M	28	2	33
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	29	2	33
Sonora	Parents 25 °C	Larva 36 °C	36 °C	F	30	3	11
Sonora	Parents 36 °C	Larva 25 °C	36 °C	M	31	2	44
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	32	3	7
Sonora	Parents 36 °C	Larva 25 °C	36 °C	F	33	3	20
Sonora	Parents 36 °C	Larva 36 °C	36 °C	M	34	2	40
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	35	2	47
Sonora	Parents 36 °C	Larva 36 °C	36 °C	F	36	2	40
Catalina	Parents 25 °C	Larva 25 °C	25 °C	M	13	2	54
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	14	2	42
Catalina	Parents 25 °C	Larva 25 °C	25 °C	F	15	2	57
Catalina	Parents 25 °C	Larva 36 °C	25 °C	M	16	2	31
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	17	2	54
Catalina	Parents 25 °C	Larva 36 °C	25 °C	F	18	2	46
Catalina	Parents 36 °C	Larva 25 °C	25 °C	M	19	2	39
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	20	2	42
Catalina	Parents 36 °C	Larva 25 °C	25 °C	F	21	2	42
Catalina	Parents 36 °C	Larva 36 °C	25 °C	M	22	2	32
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	23	2	32
Catalina	Parents 36 °C	Larva 36 °C	25 °C	F	24	2	12

Catalina	Parents 25 °C	Larva 25 °C	36 °C	M	1	1	30
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	2	3	4
Catalina	Parents 25 °C	Larva 25 °C	36 °C	F	3	2	5
Catalina	Parents 25 °C	Larva 36 °C	36 °C	M	4	2	19
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	5	2	53
Catalina	Parents 25 °C	Larva 36 °C	36 °C	F	6	1	39
Catalina	Parents 36 °C	Larva 25 °C	36 °C	M	7	2	30
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	8	2	30
Catalina	Parents 36 °C	Larva 25 °C	36 °C	F	9	2	6
Catalina	Parents 36 °C	Larva 36 °C	36 °C	M	10	2	20
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	11	2	20
Catalina	Parents 36 °C	Larva 36 °C	36 °C	F	12	1	59

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Table S1. *GLM* analysis of variance for components of viability and development time pupa-adult following heat shocks performed on larva after acclimation (phenotypic plasticity) and parental treatments (transgenerational effects) in *D. mojavensis* populations.

Effect	Df	Viability		Dev time PA	
		Df_{RES}	P	Df_{RES}	P
Population (Pop)	1	155	0.907	122	0.290
Heat shock period (HS)	1	154	0.911	121	0.771
Acclimation parents (Accl_parents)	1	153	0.932	120	0.218
Acclimation larva (Accl_larva)	1	152	0.994	119	***0.035***
Pop * HS	1	151	0.872	118	0.297
Pop * Accl_parents	1	150	0.896	117	0.231
Pop * Accl_larva	1	149	0.955	116	0.658
HS * Accl_parents	1	148	0.954	115	0.813
HS * Accl_larva	1	147	0.781	114	0.414
Accl_parents * Accl_larva	1	146	0.966	113	0.468
Pop * HS * Accl_parents	1	145	0.83	112	0.326
Pop * HS * Accl_larva	1	144	0.94	111	0.666
Pop * Accl_parents * Accl_larva	1	143	0.851	110	0.544
HS * Accl_parents * Accl_larva	1	142	0.904	109	0.243
Pop * HS * Accl_parents * Accl_larva	1	141	0.904	108	0.817

Significant values are highlighted in bold

Table S2. Complete *GLM* analysis of variance for heat knockdown in adults, including acclimation at larva and adult stages (phenotypic plasticity) and parental treatments (transgenerational effects) 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***
*Pop * Accl$_{adults}$*	1	425	***0.018***
*Pop * Accl$_{parents}$*	1	424	0.710
*Pop * Accl$_{larva}$*	1	423	0.462
*Pop * Sex*	1	422	0.744
*Accl$_{parents}$ * Accl$_{adults}$*	1	421	0.968
*Accl$_{adults}$ * Accl$_{larva}$*	1	420	0.324
*Accl$_{adults}$ * Sex*	1	419	***0.035***
*Accl$_{parents}$ * Accl$_{larva}$*	1	418	0.661
*Accl$_{parents}$ * Sex*	1	417	0.545
*Accl$_{larva}$ * Sex*	1	416	0.441
*Pop * Accl$_{parents}$ * Accl$_{adults}$*	1	415	0.717
*Pop * Accl$_{adults}$ * Accl$_{larva}$*	1	414	0.451
*Pop * Accl$_{adults}$ * Sex*	1	413	0.327
*Pop * Accl$_{parents}$ * Accl$_{larva}$*	1	412	0.194
*Pop * Accl$_{parents}$ * Sex*	1	411	0.968
*Pop * Accl$_{larva}$ * Sex*	1	410	0.321
*Accl$_{parents}$ * Accl$_{adults}$ * Accl$_{larva}$*	1	409	0.971
*Accl$_{parents}$ * Accl$_{adults}$ * Sex*	1	408	0.567
*Acclim adults * Accl$_{larva}$ * Sex*	1	407	0.854
*Accl$_{parents}$ * Accl$_{larva}$ * Sex*	1	406	0.682
*Pop * Accl$_{parents}$ * Accl$_{adults}$ * Accl$_{larva}$*	1	405	0.441
*Pop * Accl$_{parents}$ * Accl$_{adults}$ * Sex*	1	404	0.798
*Pop * Accl$_{adults}$ * Accl$_{larva}$ * Sex*	1	403	0.677
*Pop * Accl$_{parents}$ * Accl$_{larva}$ * Sex*	1	402	0.850
*Accl$_{parents}$ * Accl$_{adults}$ * Accl$_{larva}$ * Sex*	1	401	0.684
*Pop * Accl$_{parents}$ * Accl$_{adults}$ * Accl$_{larva}$ * Sex*	1	400	0.855

Significant values are highlighted in bold

Table S3. Parameter values used for individual-based simulations of *WGP* and *TGP*. These parameters correspond to results of simulations as obtained for Figure 4 in the main text as well as descriptions provided in the supplementary description of simulations.

Parameter	Value	Description
N_{ad}	5000	Maximum number of adults
N_{juv}	5000	Maximum number of juveniles
n_g	10	Number of gene loci coding for a
n_b	1	Number of gene loci coding for b
n_m	1	Number of gene loci coding for m
$\tau_{ad}\ell$	28	Adult maximal lifespan (days)
$\tau_{lv}\ell$	22,23	Juvenile lifespan (from egg to first reproduction)
μ	0.01	Mutation rate
w_{min}	0.5	Minimal survival probability
σ_e^2	0.1	Developmental noise
σ_v^2	4×10^{-4}	Mutational variance
ω_z^2	1, 10, 100	Strength of temperature-dependent selection
ω_b^2	10	Strength of selection against plasticity
ω_b^2	10	Strength of selection against maternal effects
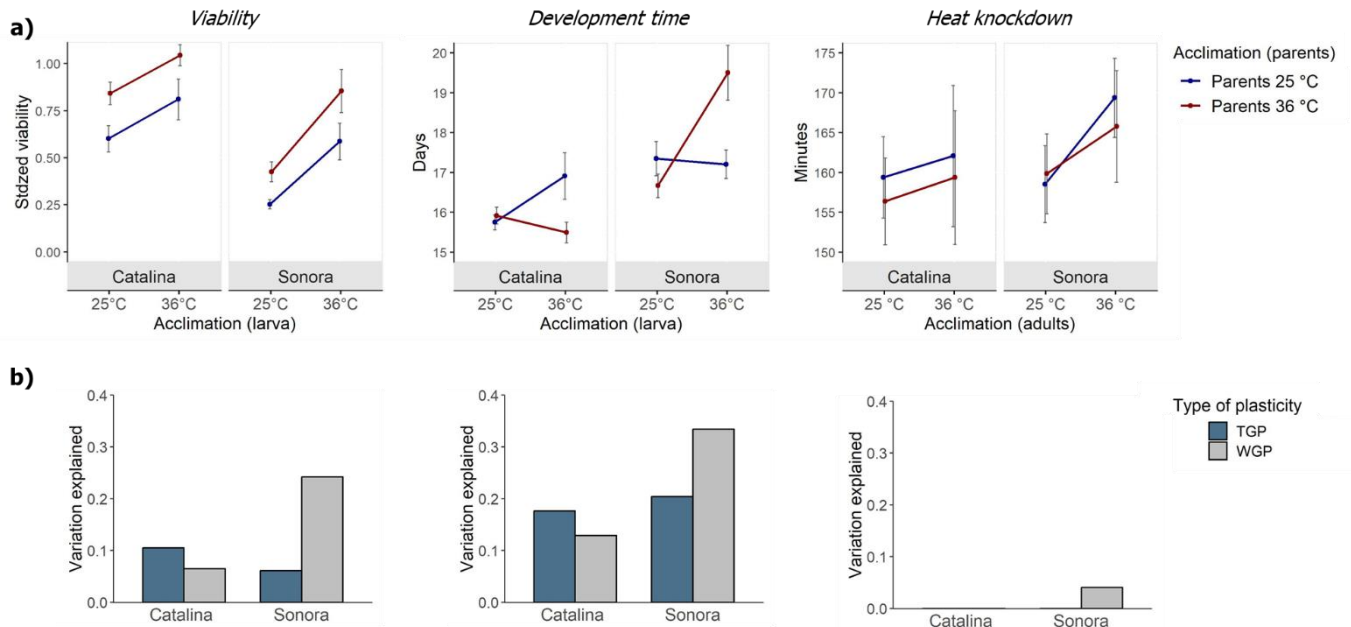
Figure S1. Heat-shock tolerance of *D. mojavensis* populations (Catalina vs Sonora) as response to heat ramping treatments (up to 40°C) following acclimation treatments performed in parents and offspring generations (25 vs 36°C). Heat shocks were performed using a ramping treatment between 30°C to 40°C at 0.13°C/min followed by 1.5h at 40°C for experiments in larvae or until reaching knockdown for experiments in adult females. First panel **a)** represents results obtained for viability larva-adult (standardized), development time larva-adult and heat knockdown (± SE). Second panel **b)** represents results of variation partitioning analysis, with the proportion of variation explained by transgenerational plasticity (*TGP*) and within-generation plasticity (*WGP*) with respect to total variation for each trait and population. Only results for 1.5h heat-shocks in larvae and adult males are shown. Results for 2h heat-shocks and adult females are included in Figure 2.
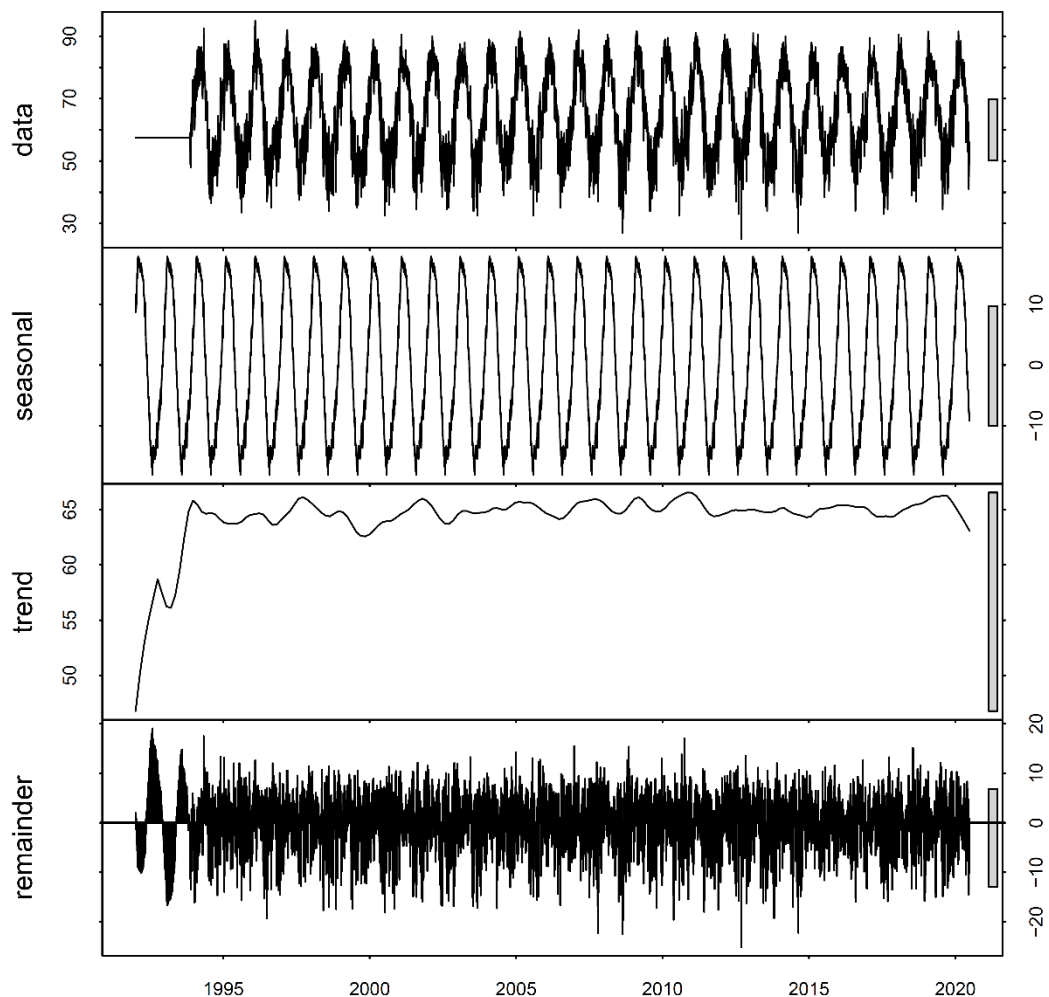
Figure S2. Loess decomposed time series of the average temperature in Sonora, using *stats::stl()* in R. Individual-based simulations are based on the seasonal component (2nd row) (Data provided by National Centers for Environmental Information, National Oceanic and Atmospheric Administration – NOAA from their web site https://www.ncdc.noaa.gov/cdo-web/datasets#NORMAL_HLY).
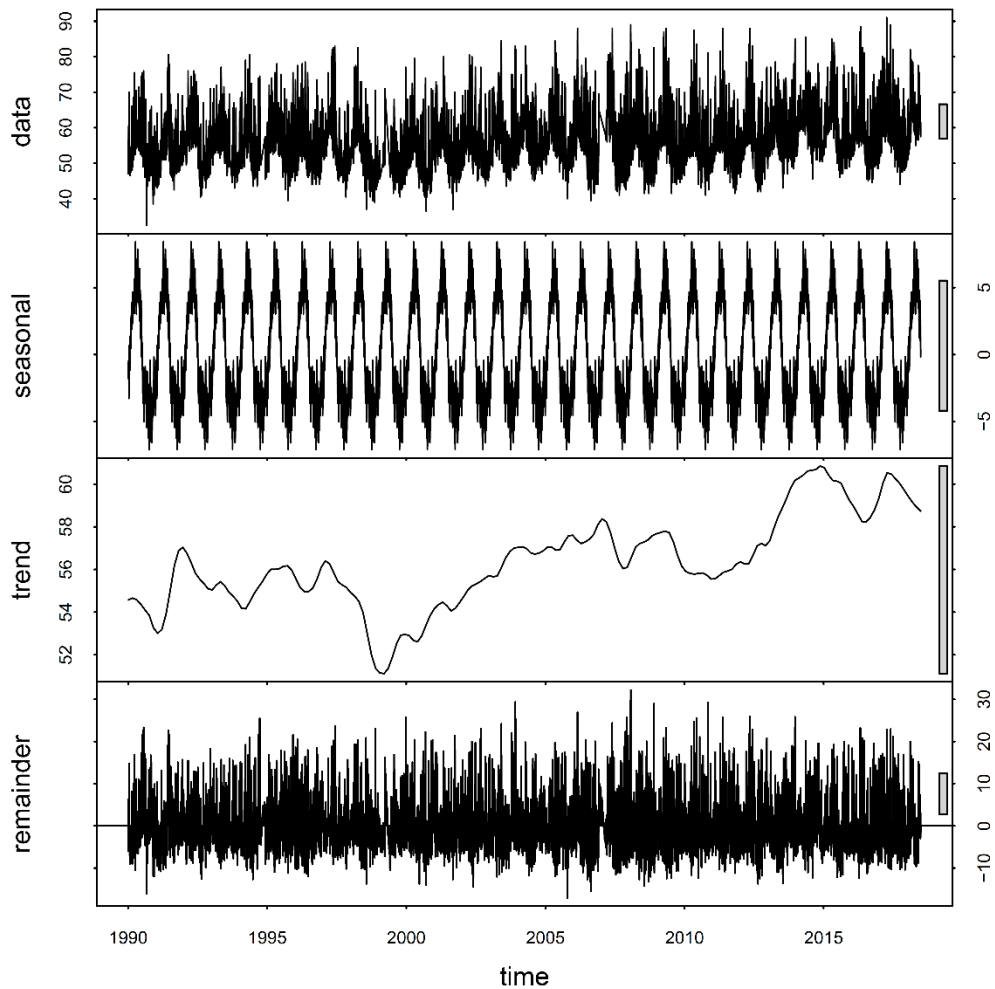
Figure S3. Loess decomposed time series of the average temperature in Catalina, using *stats::stl()* in R. Individual-based simulations are based on the seasonal component (2nd row) (Data provided by National Centers for Environmental Information, National Oceanic and Atmospheric Administration – NOAA from their web site https://www.ncdc.noaa.gov/cdo-web/datasets#NORMAL_HLY).
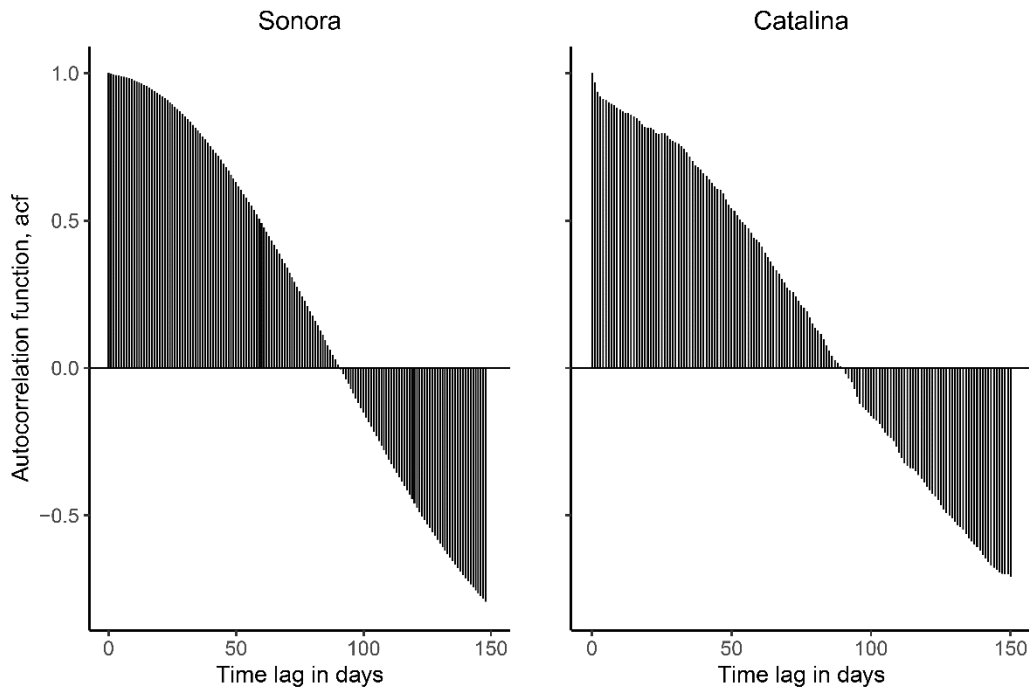
Figure S4. Autocorrelations of the seasonal temperature component versus time lag in days for Sonora (panel A) and Catalina (panel B). Overall, there is little difference in autocorrelation structure across both locations. Also note that the maximum lifespan of an individual is 51 days, so that the parental and offspring environments are typically positively autocorrelated. Autocorrelations are taken over seasonal data obtained from the second rows of Figures S2 and S3, after standardization using the overall mean and variance in temperature (i.e., across both locations). Each horizontal line depicts the correlation of the seasonal temperature component on day τ with temperature on day $\tau + I$ (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).
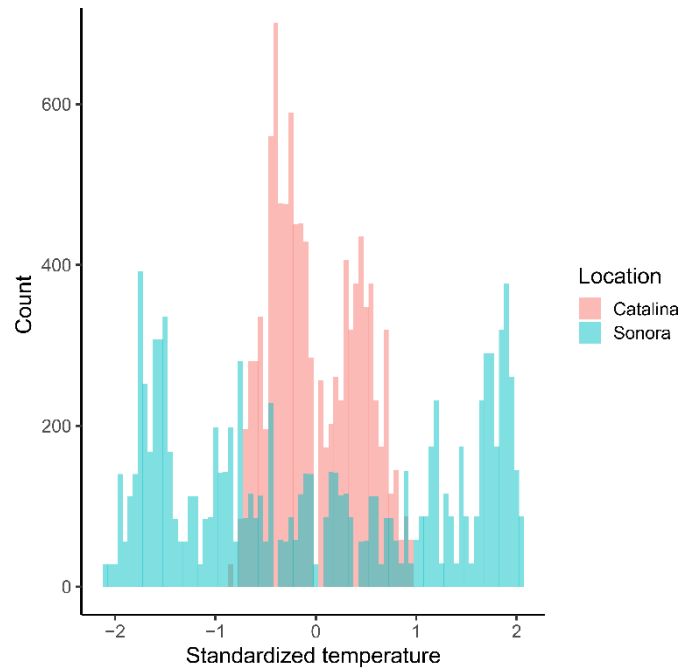
Figure S5. Histograms comparing the range of standardized temperatures across both locations. Note that temperature variance is much larger in Sonora than in Catalina (Data provided by National Centers for Environmental Information, National Oceanic and Atmospheric Administration – NOAA from their web site https://www.ncdc.noaa.gov/cdo-web/datasets#NORMAL_HLY).
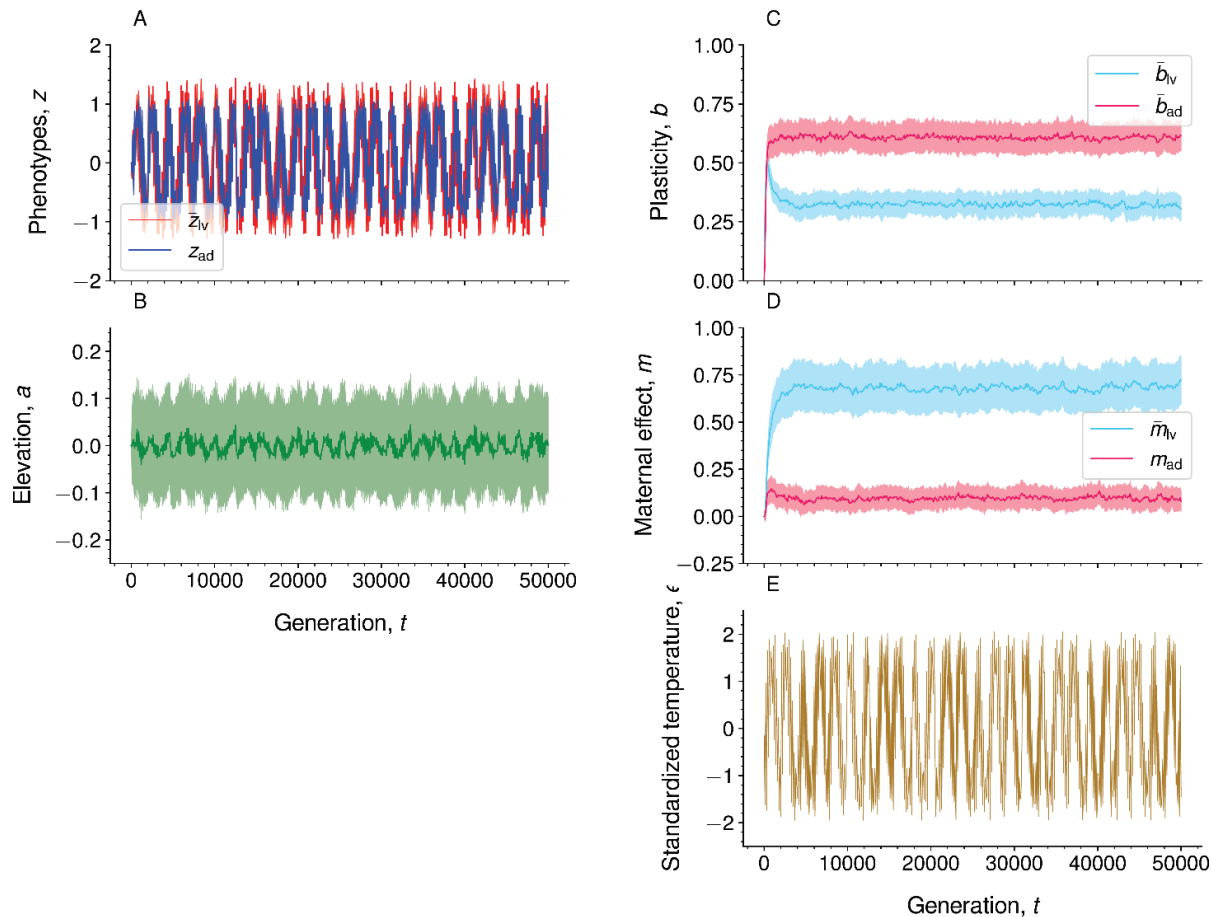
Figure S6. Evolving phenotypes over time for a single example individual-based simulation in Sonora. Panel A: average phenotypes expressed in adults ($\bar{z}_{ad}$ blue) and larvae ($\bar{z}_{lv}$, red). Panel B: average genetic elevation $\bar{a}$. Panel C: within-generational plasticity expressed in adults ($\bar{b}_{ad}$, blue) and larvae ($\bar{b}_{lv}$, pink). Panel D: transgenerational plasticity expressed in adults ($\bar{m}_{ad}$, blue) and larvae ($\bar{m}_{lv}$, pink). Panel E: the standardized temperature. To reduce image size, all values are plotted at 50 generation intervals. Each generation lasts 50 days. Parameters as in Figure 3.
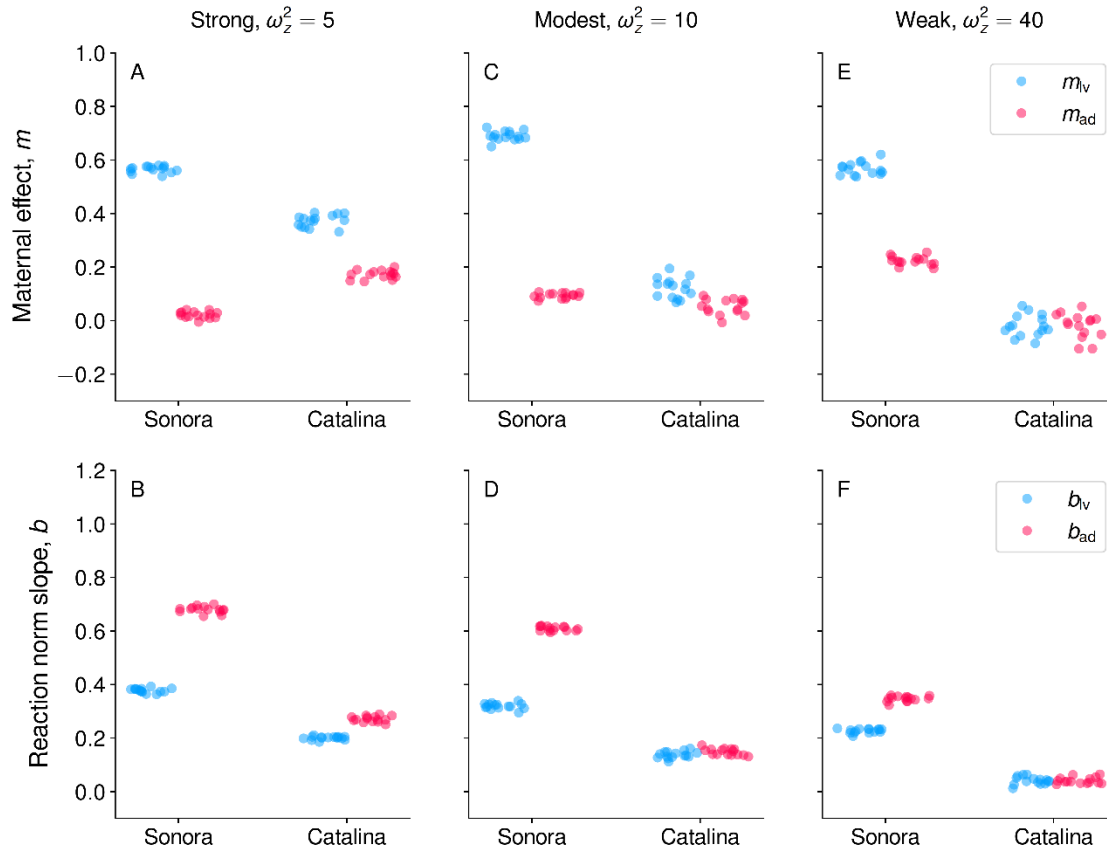
Figure S7. Evolved values of transgenerational and within-generational plasticity after 50,000 generations when varying the overall strength of selection ω^2_z from strong to weak. For the corresponding *WGP* and *TGP* reaction norms, see Figure 3 in the main text (for $\omega^2_z = 10$) and Supplementary Figures S9 and S10 (for larval traits only; adult traits not shown for the sake of brevity). Each dot reflects the evolved average value of from a single replicate simulation. Panels A, C, E: maternal effects that affect offspring as larvae (m_{lv}: blue dots) and as adults (m_{adult}: red dots). Panels B, D, F: within-generational plasticity that affects offspring as juveniles (b_{lv}: blue dots) and as adults (b_{ad}: red dots). Typically, both within-generational plasticity and transgenerational plasticity have evolved to attain larger values in Sonora than in Catalina. Next, note that maternal effects that affect traits in adult flies (m_{ad}) are generally smaller than maternal effects that affect traits in larvae (m_{lv}). By contrast, within-generational plasticity is typically stronger in adults (b_{ad}) than in larvae (b_{lv}). For adult flies, maternal phenotypes are less informative as the environment has changed since the time of birth, thus selectively favoring phenotypic plasticity over maternal effects. Parameters: $\omega_{min} = 0.5$, $\sigma^2_e = 0.1$.
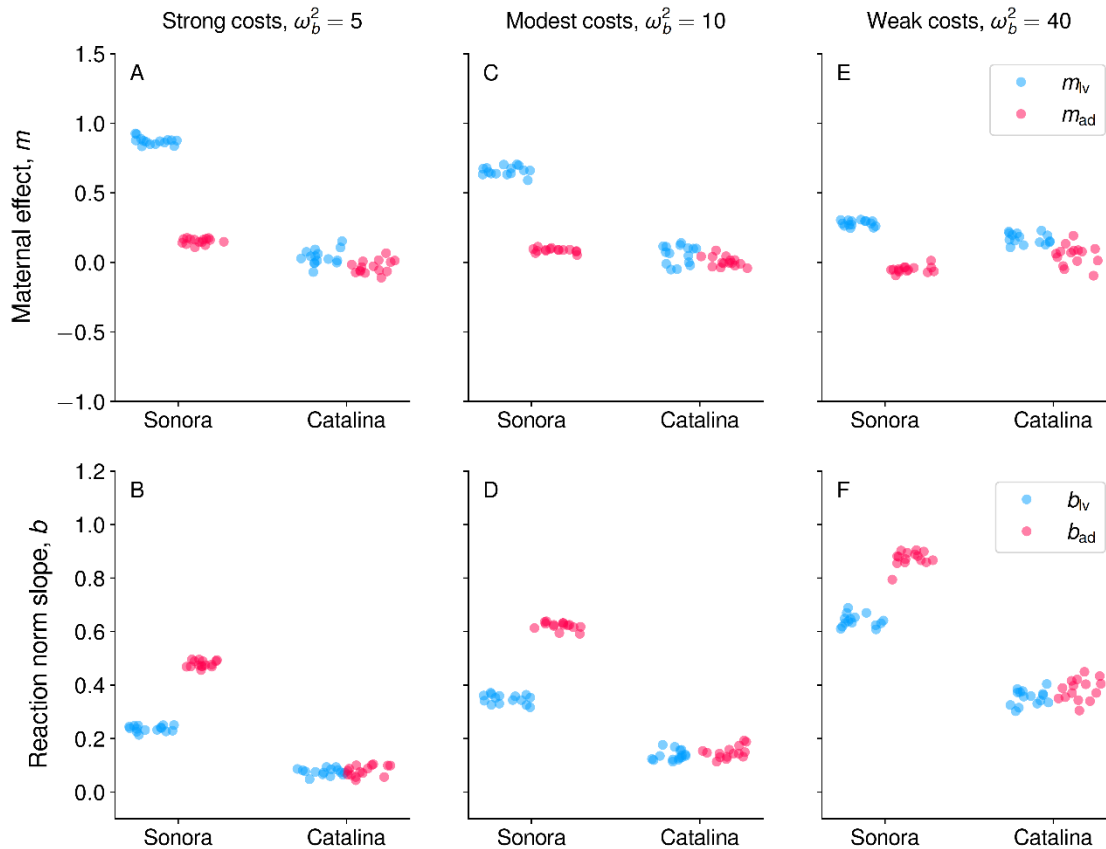
Figure S8. Evolved values of transgenerational and within-generational plasticity after 50,000 generations when varying the costs of within-generational plasticity ω^2_b from strong to weak. For the corresponding *WGP* and *TGP* reaction norms, see Figure 3 in the main text (for $\omega^2_b = 10$) and Supplementary Figures S9 and S10 (for larval traits only; adult traits not shown for the sake of brevity). Each dot reflects the evolved average value of from a single replicate simulation. We find that with increasing costs of within-generational plasticity, the values of the within-generational reaction norm slopes b_{lv}, bad become smaller, while mlv and mad become larger. Regardless, both *WGP* and *TGP* evolve to attain stronger values in Sonora relative to Catalina. Parameters: $\omega_{min} = 0.5$, $\sigma^2_e = 0.1$.
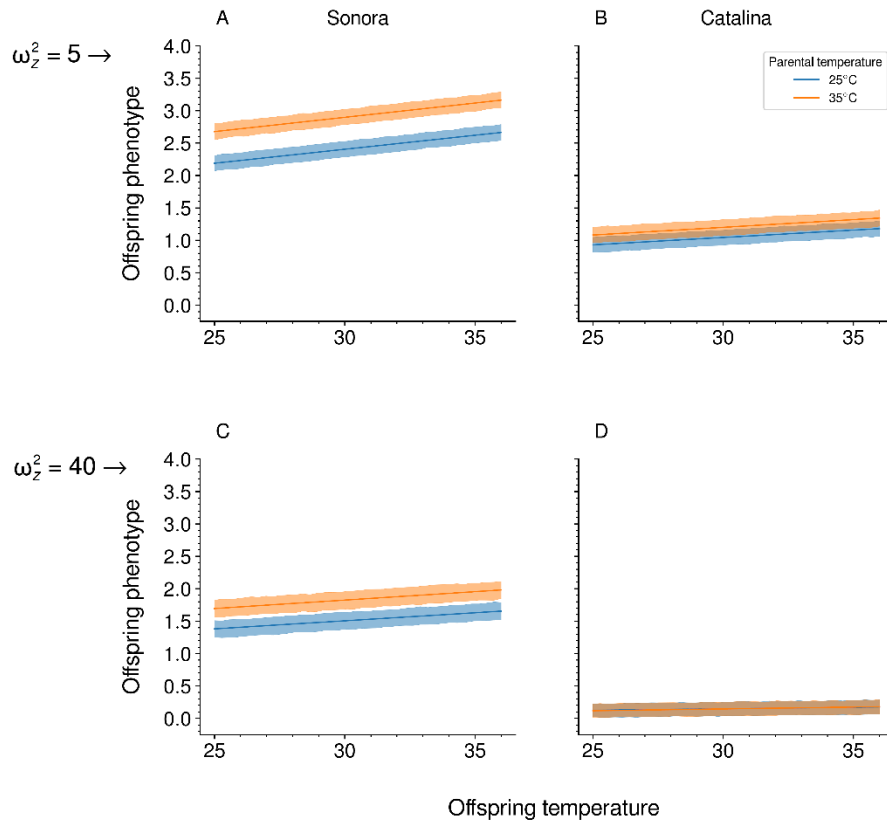
Figure S9. Reaction norms affecting larval traits, based on the evolved values of a, b_{lv}, b_{ad}, m_{lv}, m_{ad} from the individual-based simulations, while varying the strength of selection ω^2_z. Also, for different strengths of selection, we find that *WGP* and *TGP* evolve to attain larger values in Sonora, relative to Catalina. Panels A, B: Strong selection where $\omega^2_z = 5$. Panels C, D: Weak selection where $\omega^2_z = 40$. See Figure 3 in the main text for parameter values.
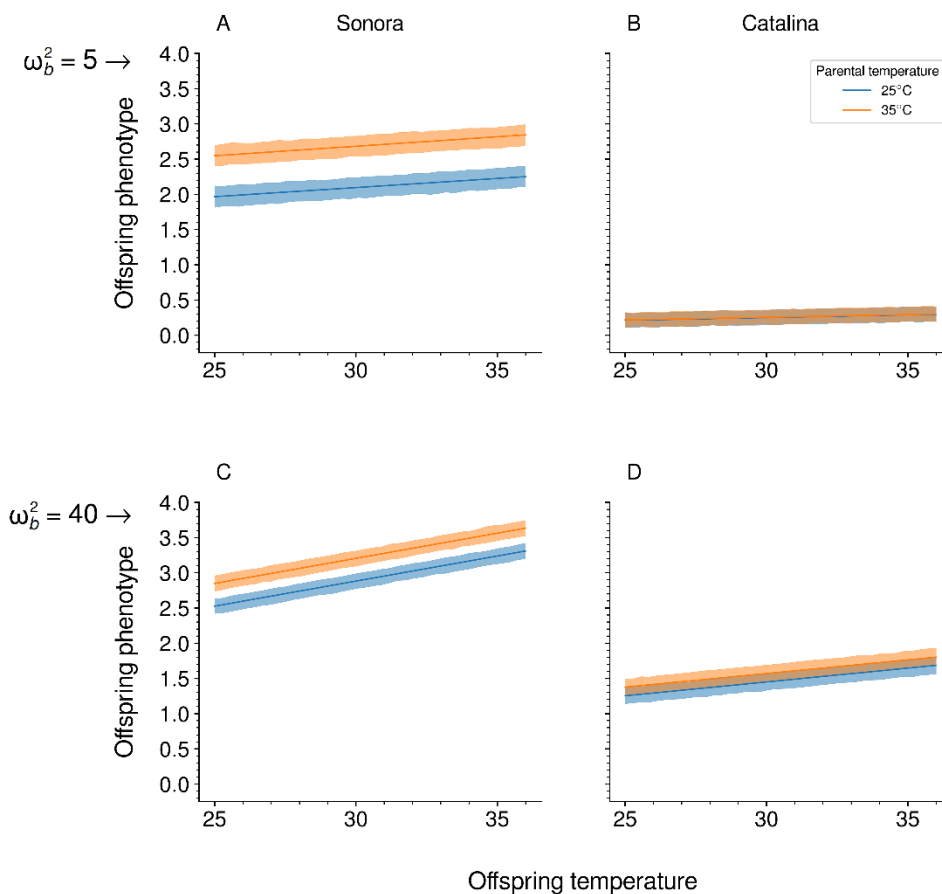
Figure S10. Reaction norms affecting larval traits, based on the evolved values of a, b_{lv}, b_{ad}, m_{lv}, m_{ad} from the individual-based simulations, while varying the cost of *WGP* ω^2_b. Similar to Figures 3 and S8 we find that *WGP* and *TGP* evolve to attain larger values in Sonora, relative to Catalina. See Figure 3 in the main text for parameter values.