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Stage- and thermal-specific genetic architecture for preadult viability in natural populations of *Drosophila melanogaster*

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Abstract

Studying the processes affecting variation for preadult viability is essential to understand the evolutionary trajectories followed by natural populations. This task requires focusing on the complex nature of the phenotype–genotype relationship by taking into account usually neglected aspects of the phenotype and recognizing the modularity between different ontogenetic stages. Here, we describe phenotypic variability for viability during the larval and pupal stages in lines derived from three natural populations of *Drosophila melanogaster*, as well as the variability for phenotypic plasticity and canalization at two different rearing temperatures. The results indicate that the three populations present significant phenotypic differences for preadult viability. Furthermore, distinct aspects of the phenotype (means, plasticity, canalization, plasticity of canalization) are affected by different genetic bases underlying changes in viability in a stage- and environment-specific manner. These findings explain the generalized maintenance of genetic variability for this fitness trait.

KEYWORDS

Adaptation, canalization, development, evolvability, genetic architecture, plasticity, viability

1 | INTRODUCTION

The study of fitness-related traits provides a powerful tool to describe the organismal investment of resources in different biological functions, underlying particular adaptive strategies that depend on the ecological scenario (van Noordwijk & de Jong, 1986; Stearns, 1992). In this sense, elucidating the genetic architecture for developmental traits affecting fitness allows the characterization of the processes that lead to phenotypic diversity and determine evolutionary potential (Hansen, 2006).

Although the traditional framework for studying the relationships between genotype and phenotype focuses on traits' means in certain environments, aspects relating to phenotypic variability are equally relevant to the understanding of developmental processes underlying ecological strategies and phenotypic evolution (Ørsted, Rohde, Hoffmann, Sørensen, & Kristensen, 2018; Paaby & Testa, 2018). One such parameter is phenotypic plasticity, the ability of a genotype to modify the phenotype according to the environment (DeWitt, 2016), which constitutes a strategy to deal with environmental heterogeneity (DeWitt & Scheiner, 2004): when phenotypic optimum for a given trait varies across environments frequently experienced by a population, plasticity for the trait is adaptive and is expected to arise (DeWitt & Scheiner, 2004). This phenomenon also implies that the effect of some genetic variants can be concealed to natural selection in some conditions, favouring the accumulation of genetic variability (Paaby & Gibson, 2016).

Phenotypic variation within environments, which has been shown to have a genetic basis (Blasco, Martínez-Álvaro, García, Ibáñez-Escriche, & Argente, 2017; Ørsted, Rohde, Hoffmann, Sørensen & Kristensen, 2018; Sørensen, de los Campos, Morgante, Mackay, & Sorensen, 2015), is also relevant to the potential evolutionary trajectory of populations. In fluctuating environments,

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genetic variants that favour a high phenotypic variance may maximize fitness; this phenomenon is known as "bet hedging strategy." Conversely, in constant environments, stabilizing selection would promote the fixation of epistatic interactions promoting the robustness of phenotype to microenvironmental or genetic changes (environmental or genetic canalization, respectively [Debat & Le Rouzic, 2019; Ørsted et al., 2018]). It has been proposed that traits with a great impact on fitness are usually more strongly canalized (Stearns & Kawecki, 1994). Phenotypic canalization hides the effect of some genetic variants from natural selection, and thus, these variants are less likely to be selected against. Under subsequent genetic or environmental perturbation, a decanalization of the phenotype and the liberation of cryptic genetic variation as additive genetic variance may occur, with the possibility of rapid phenotypic evolution (Flatt, 2005; Paaby & Gibson, 2016). Although genetic canalization refers to genetic variance within a population, the degree of environmental canalization for a given trait, that is the genotypes' ability to produce similar phenotypes within an environment, can be estimated by the environmental variation coefficient (CVE), which standardizes environmental variability according to phenotypic means. Estimating CVEs allows for the comparison of environmental canalization between different populations or even different traits. Environmental canalization may be plastic, meaning the phenotypic robustness across environments for a given genotype is not constant (Ørsted et al., 2018). Phenotypic plasticity, canalization and the plasticity of canalization for a trait emerge from its genetic architecture and are important for the maintenance of genetic variability, invasiveness and evolvability (DeWitt, 2016; Gilchrist & Lee, 2007; Ørsted et al., 2018; Paaby & Gibson, 2016). Relationships between phenotypic means and these components of environmental variation are apparently complex, and they may depend on the assessed trait and the range of environments (Blasco et al., 2017; Harbison, McCoy, & Mackay, 2013; Morgante, Sørensen, Sorensen, Maltecca, & Mackay, 2015; Reed et al., 2010; Sørensen et al., 2015).

Preadult viability, the proportion of individuals reaching maturity, is a trait with a direct effect on fitness (Fowler, Semple, Barton, & Partridge, 1997; Futuyma, 1998) and is correlated with other fitness-related traits, such as developmental time, pupation behaviour or resistance to different stressful conditions (Casares & Carracedo, 1987; Chippindale, Chu, & Rose, 1996; Folguera, Ceballos, Spezzi, Fanara, & Hasson, 2008; Prasad, Shakarad, Anitha, Rajamani, & Joshi, 2001; Prasad et al., 2000). Differences among and within populations reared in the same environmental conditions reveal genetic variation for this trait (Folguera et al., 2008; Gardner, Fowler, Partridge, & Barton, 2001; Horváth & Kalinka, 2016; Mackay, 1986; Rodríguez-Ramilo, Pérez-Figueroa, Fernández, Fernández, & Caballero, 2004) that can be maintained by genotype-environment interaction (Fanara, Folguera, Fernandez Iriarte, Mensch, & Hasson, 2006; Horváth & Kalinka, 2016).

The traditional framework evaluates preadult viability as the survival from the first instar larva until the emergence of the imago. However, both larval and pupal ontogenetic stages present anatomically, physiologically and behaviourally dissimilar characteristics, so that they could be analysed as distinct modules. It has been proposed that the modularity between life stages in holometabolous insects results in a greater intra- and interspecific diversity (Minelli, Brena, Deflorian, Maruzzo, & Fusco, 2006; Yang, 2001). Furthermore, modularity at different levels (e.g. genetic, anatomical) would restrict the impact of changes in one module over the rest, thus preventing the production of trade-offs (patterns of negatively correlated fitness effects) that act as evolutionary constraints (Chippindale et al., 1996; Hughes & Leips, 2017).Therefore, modularity would generally favour the maintenance of genetic variability and increase evolvability (Hansen, 2003; Hill & Zhang, 2012; Wagner & Zhang, 2011) and should be accounted for when studying developmental fitness traits (Bolker, 2000; Hoekstra & Coyne, 2007). For that reason, the decomposition of preadult viability in its larval and pupal components would be of interest not only to achieve a higher resolution when describing phenotypic variability but also to provide relevant information on the degree of modularity at the ontogenetic level.

In this work, we expect to characterize phenotypic variability for preadult viability and to demonstrate the hypothesis that its genetic architecture can change in response to environmental conditions and ontogenetic stage. Furthermore, we wish to determine whether the different aspects of phenotype behave independently and therefore can respond to different selective pressures and generate complex ecological strategies. With these objectives, we analysed the variability for Larval and pupal viability (LV and PV) means, their plasticity and canalization patterns at two different rearing temperatures (17° and 25°C) in isogenic lines derived from three natural populations of *Drosophila melanogaster*. This setting allowed us to i) test if there is phenotypic variability for viability traits (means and variances) between and within populations; ii) estimate the weight of genetic and environmental components underlying this variation; iii) test if the genetic architecture for viability changes along ontogeny; and iv) test if the genetic bases for means and environmental variances are decoupled.

Our results show differences between populations for both traits, their canalization patterns and plasticities. The genetic bases for these different aspects of phenotype were apparently decoupled, suggesting the possibility for their independent evolution and the subsequent emergence of distinct ecological strategies. A genotype-environment interaction was observed for LV and PV in the three populations, with a change in genotypic ranking across environments, contributing to the maintenance of genetic variability. Finally, our results point towards a differentiated genetic basis for viability not only between populations, but also according to developmental stage and rearing temperature. We therefore argue for a comprehensive characterization of phenotypic variability, including the study of environmental variance components, to achieve a more realistic view of the ecological strategies and evolutionary dynamics followed by natural populations and to understand changes in their ability to evolve.

2 | MATERIALS AND METHODS

2.1 | Populations

Three different populations of *D. melanogaster* were used. A subset of lines corresponds to the *Drosophila Genetic Reference Panel* (DGRP), derived from mated females collected in North Carolina (Mackay et al., 2012). The other two populations were obtained from flies collected in Lavalle and Uspallata (Province of Mendoza, Argentina). The choice of Argentinean populations was based on their locations of origin on both extremes of an altitudinal cline, and therefore their different thermal regimes. Relevant geographic and climatological information corresponding to the three populations is listed in Table 1. Thermal amplitude was estimated as the difference between maximal and minimal monthly averages; daily thermal amplitude was calculated by averaging the difference between each month maximal and minimal temperatures. Data assigned to Lavalle correspond to the nearby location of Jocolí Viejo. Climatological data were obtained at http://es.climate-data.org/. Grapevine, plum, cantaloupe and peach were grown in the region of the Lavalle collection site, whereas Uspallata lines were collected at an apple, pear and quince plantation. *D. simulans* individuals were also collected in both locations, whereas *D. buzzatii* specimens were only found in Lavalle.

Isogenic lines were generated from mated females collected in the Argentinian locations following the protocol performed by Mackay et al. (2012). Isofemale lines were established from gravid females collected in Uspallata and Lavalle, and they were after inbred by 20 generations of full-sib mating, followed by random mating, obtaining lines with an estimated inbreeding coefficient of 0.986 (Mackay et al., 2012). Afterwards, each line was maintained independently. All lines were kept in laboratory (cornmeal–agar–yeast) medium in an incubator at 25 ± 1°C, under a 12-hr light : 12-hr dark cycle and at 70% humidity. 40 lines from Raleigh, 34 lines from Lavalle and 34 lines from Uspallata were measured.

Microsatellite analyses (Goldstein & Pollock, 1997) conducted in the Lavalle and Uspallata populations allowed us to discard the possibility of recent demographic events such as reduction in population sizes (Ortiz & Satorre, personal communication).

2.2 | Viability assays

For each line of each population, large quantities of first instar larvae were obtained by placing batches of 100 pairs of sexually mature flies into egg-collecting chambers with Petri dishes containing an agar and yeast egg-laying medium (three chambers for each line). After larvae started hatching, batches of 30 first instar larvae were transferred from the Petri dishes to culture vials containing 5 ml of cornmeal–agar–molasses; this density prevents crowding effects. Vials from each line were kept in an incubator at two temperatures: (17 ± 1)°C and (25 ± 1)°C, respectively, representing the mean temperatures for summer months in the highland and lowland locations considered, under a 12-hr light : 12-hr dark cycle and at 70% humidity. We set up 4–5 replicates for each line–temperature combination, representing a total of 952 vials. Every 24 hr, new pupae were counted and emerged flies were collected.

For each replicate, LV was estimated as the percentage of pupae formed from the total of first instar larvae initially seeded (30), whereas PV was estimated as the proportion of hatched adults from the number of formed pupae. We also calculated, for each line–temperature combination, CVEs for viability traits as the ratio between standard deviation and average for the replicates.

Phenotypic plasticities for all traits' means corresponded to the quotient of the means per line at 17°C and 25°C, whereas plasticities for CVEs were calculated by subtracting CVEs by line at 25°C from CVEs by line at 17°C.

The differences between populations for both viability traits were evaluated using the averages by line and temperature by means of two-way fixed model analysis of variance (ANOVAs) according to the model

$$y = \mu + P + T + P \times T + \varepsilon$$

where P and T are the fixed effects of population and temperature, and ε stands for the error (variance within $P \times T$ combination). The population effect tests for phenotypic differentiation among populations, whereas $P \times T$ stands for variation in thermal plasticity among populations. When $P \times T$ interaction was significant, partial ANOVAs by temperature were carried out.

The variance for viability traits within populations was evaluated using the averages by replicate, by means of two-ways analysis of variance (ANOVAs) for each population, according to the mixed model

$$y = \mu + L + T + L \times T + \varepsilon$$

where L and T are the effects of line (random) and temperature (fixed), and ε stands for the error (variance within $L \times T$ combination). In these ANOVAs, significant L and $L \times T$ effects are interpreted as the presence of genetic variation and genetic variation in thermal

TABLE 1 Collection sites and selected climatological data for the three populations of *Drosophila melanogaster* utilized in this study: Raleigh (North Carolina, USA), Lavalle (Mendoza, Argentina) and Uspallata (Mendoza, Argentina). Altitude is given in metres; all temperature information is in °C, and precipitation is expressed in millimetres

			Annual Temperature		Daily thermal	Annual
Population	Location	Altitude	Average	Min–Max	Amplitude	Precipitations
Raleigh	35°46′N, 78°38′W	115	15.3	6.8–25.8	12.3	1147
Lavalle	32°30′S, 68°58′W	580	17.3	7.7–25.1	14.7	175
Uspallata	32°35′S, 69°22′W	1915	10.5	4.6–17.1	14.8	156

plasticity, respectively. A significant $L \times T$ effect, an estimator of the genotype-environment interaction, can arise as a consequence of differences in among-lines variance in separate environments (change in scale) and/or deviations from unity of the cross-environment genetic correlation (change in ranking order). The contribution of the two sources of variation to genotype-environment interaction was analysed by means of the equation (Muir, Nyquist, & Xu, 1992; Robertson, 1959):

$$V_{GEI} = [(\sigma_{17^\circ C} - \sigma_{25^\circ C})^2 + 2 \times \sigma_{17^\circ C} \times \sigma_{25^\circ C} \times (1 - rG \times E_{(17^\circ C\ 25^\circ C)}]/2$$

where V_{GEI} is the genotype-environment interaction variance component, $\sigma_{17^\circ C}$ and $\sigma_{25^\circ C}$ are the square roots of the among-lines variance components at 17°C and 25°C (which were obtained after performing ANOVAs for each temperature separately), and $rGxE_{(17^\circ C\ 25^\circ C)}$ is the cross-environment genetic correlation. $rGxE_{(17^\circ C\ 25^\circ C)}$, which reflects the degree in which the same genetic variants control trait expression across temperatures, was estimated for each trait as $COV_{17^\circ C\ 25^\circ C} / \sigma_{17^\circ C}\ \sigma_{25^\circ C;}$ where $COV_{17^\circ C\ 25^\circ C}$ represents the covariance of lines means measured at 17°C and 25°C and, $\sigma_{17^\circ C}$ and $\sigma_{25^\circ C}$ were defined above. This method for the estimation of $rGxE_{(17^\circ C\ 25^\circ C)}$ is not equivalent to the computation of a product-moment correlation (Lynch & Walsh, 1998).

Quantitative genetic parameters were calculated for both larva and pupal viability in each population. Under our experimental design, genetic (σ^2_G), environmental (σ^2_E) and phenotypic (σ^2_P) variance was estimated as $\sigma^2_G = \sigma^2_L + \sigma^2_{LT}$; $\sigma^2_E = \sigma^2_W$ and $\sigma^2_P = \sigma^2_G + \sigma^2_E$, where σ^2_L, σ^2_{LT} and σ^2_W are among-line, line by temperature and within-line variance components, respectively. Broad sense heritability (H^2) for each trait was estimated as $H^2 = \sigma^2_G/\sigma^2_P$. Coefficients of genetic ($CV_G = 100\sigma_G/$mean) and environmental ($CV_E = 100\sigma_E$/mean) variance were also computed. We also estimated H^2_i for each combination of population and temperature, as $\sigma^2_{Li}/(\sigma^2_{Li} + \sigma^2_{Wi})$, where σ^2_{Li} and σ^2_{Wi} are the among-line and within-line variance components obtained from the one-way ANOVA for each population performed for 17°C and 25°C. Correlation analyses were carried out between LV and PV within each temperature, between temperatures for each V trait, between viability traits and their CVEs and between phenotypic (mean) plasticity and CVE plasticity for each population.

All statistical tests were performed using STATISTICA 8.0 (StatSoft, Inc). Bonferroni correction for multiple tests was applied whenever results from multiple tests were combined in one final conclusion.

3 | RESULTS

We scored a total of 18,322 adults derived from 21,408 pupae that developed from ~30,000 first instar larvae. The overall viability considering all populations was 61.1%, being the larval and pupal viabilities 71.4% and 85.6%, respectively. The populations did not differ significantly in the number of replicates excluded and no lines showed more than 1 replicate for each temperature with a total viability <25% (data not shown).

Correlation analyses (Table 2) revealed no significant associations between LV and PV in any of the three populations at either of the rearing temperatures. Also, our results showed no significant correlations between temperatures for any of the preadult viability traits. These results suggest that the genetic bases for LV and PV were decoupled and that different genetic factors were associated to development at different temperatures in both viability traits. Thus, we performed the analyses for LV and PV separately.

Our data showed a higher PV than LV at both temperatures with the sole exception of the Raleigh population at 17°C (Figure 1), although when considering the average duration of each stage, hourly mortality was higher for the three populations at 17°C and during the pupal stage (Figure S1). Furthermore, both mean viabilities and CVEs exhibited a greater dispersion at 25°C compared to the results observed at 17°C. On the other hand, distinct patterns were found for LV and PV, as expected by the lack of statistical correlations between both traits. We detected significant variation among populations only for LV (Table 3a), where the higher average viability corresponded to lines collected in Raleigh population. The populations' mean viabilities responded to thermal variation differently (Table 3a, Figure 1a). Argentinean populations exhibited a similar pattern for LV means and CVEs, with higher viabilities at 17°C; LV for Raleigh population showed less plasticity. Conversely, for both means and CVEs of PV, lines from the Raleigh population were the most plastic on average, whereas those from Uspallata had the most constant phenotype across temperatures (Figure 1), in a pattern that mirrored the annual thermal amplitudes of the original locations. On the other hand, CVEs of LV did not change according to temperature (Table 3), adding evidence for the decoupling between both components of preadult viability.

To further evaluate the phenotypic differences between the three populations, we performed ANOVAs within each population

TABLE 2 Pearson's correlations between larval viability (LV) and pupal viability (PV) among isogenic lines derived from Raleigh (R), Lavalle (L) and Uspallata (U). *r*-values and *p*-values are shown for each trait between temperatures (in bold), between each pair of traits within 17°C (above, right) and between each pair of traits within 25°C (below, left)

	LV	PV
LV		
R	**0.19 (*p* = 0.239)**	−0.04 (*p* = 0.289)
L	**−0.14 (*p* = 0.424)**	0.11 (*p* = 0.654)
U	**0.29 (*p* = 0.096)**	0.11 (*p* = 0.681)
PV		
R	0.18 (*p* = 0.554)	**0.06 (*p* = 0.708)**
L	−0.20 (*p* = 0.652)	**−0.38 (*p* = 0.027)***[a]
U	−0.08 (*p* = 0.313)	**0.00 (*p* = 0.992)**

[a]Not significant after Bonferroni correction for multiple tests ($P_B = 0.01$).
*$p < 0.05$.

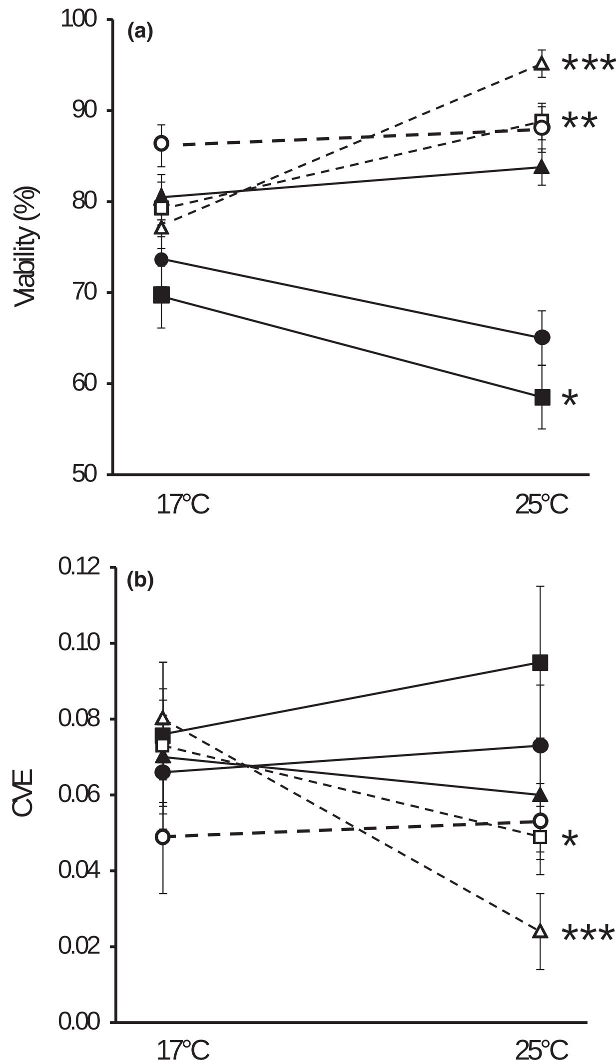

FIGURE 1 Means and CVEs of Viability for individuals reared at 17°C and 25°C from Raleigh (triangle), Lavalle (square) and Uspallata (circle). Larval viability and pupal viability are depicted by filled and open symbols, respectively. Vertical bars represent standard deviation. Asterisks represent significant differences between temperatures for the trait – population combination (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$)

to investigate whether the genetic and environmental factors affecting viability traits were homogeneous across populations and estimate their relative contributions. Results revealed significant natural genetic variation underlying variability for LV and PV in the three populations, since variation attributable to genetic factors (line and line × temperature interaction) contributed at least ~40% of the total variance (Figure 2). For all three populations and for both viability traits, the genotype-environment interaction, estimated by means of the line × temperature interaction, was always the main factor contributing to genetic variance, wherein changes in ranking order among environments accounted for at least 76% of the interaction (Table 4, Figure 3). The estimated cross-temperature genetic correlations ($rGxE_{(17°C\ 25°C)}$) for each trait and population (Table 4) serve as a measure of the extent to which the same genetic variants affect a trait in flies raised at both temperatures. This $rGxE_{(17°C\ 25°C)}$ correlation ranked from −0.53 to 0.31 determining thermal-specific differences in the genetic bases of both traits in lines collected in Raleigh, Lavalle and Uspallata. This pattern implies that optimal phenotypes at both temperatures were not associated to the same genotypes, thus limiting the loss of genetic variability in heterogeneous environments. Certainly, the analyses indicate that Raleigh, Lavalle and Uspallata populations harboured genetic variation affecting all traits: differences among lines accounted for at least 22% of the total phenotypic variance within each temperature (data not shown).

To gain insights into the differences in genetic architecture between LV and PV in all populations, we calculated quantitative genetic parameters (Table 4). The data showed moderate broad sense heritabilities for LV and higher values for PV. It is of interest to note that the values for CV_G ranged from 16.07 to 27.75 (for LV) and from 9.47 to 14.09 (for PV) for the three populations, suggesting that LV had a greater potential for change under selection, at least in the conditions assayed.

Finally, negative correlations were found between viability and CVEs as well as between plasticity for means and CVEs for PV in the three populations (Table 5). These results could suggest a common genetic basis for these different components of pupal phenotype. On the other hand, no consistent relationships were found for LV means and CVEs and between plasticities for means and CVEs, although opposing patterns for these plasticities were found in the Raleigh populations with respect to the Argentinian ones (Table 6), with a higher robustness at 25°C compared to 17°C.

4 | DISCUSSION

Studying the evolution of genetic architecture of developmental traits affecting fitness is essential to understand their evolutionary dynamics, including adaptation to different and heterogeneous environmental conditions (Hansen, 2006; Mackay, 2001; Mackay & Huang, 2018). Here, we used lines derived from three natural populations of *D. melanogaster* to characterize phenotypic variability and genetic architecture for preadult viability traits at two temperatures.

Our results revealed phenotypic differences between populations for larval and pupal viabilities, indicating changes in genetic architecture, as population-specific trends for preadult viability traits and distinct patterns of phenotypic canalization depending on the developmental temperature were found.

We also detected a high contribution of genetic components to total phenotypic variability within populations and a moderate (for larvae) to high (for pupae) heritabilities, suggesting that genetic variance for preadult viability is not exhausted by natural selection, as it would be expected for fitness traits (Falconer & Mackay, 1996; Lavagnino, Anholt, & Fanara, 2008; Mousseau & Roff, 1987). This finding can be explained by the genetic variation found for thermal reaction norms within populations (genotype-environment interaction). Indeed, several studies show that genetic variability can be maintained by genotype-environment interaction

TABLE 3 Results of the ANOVAs examining differences for (a) larval viability (LV) and pupal viability (PV) and (b) CVEs for LV and PV among Populations (P), and between Temperatures (T) wherein individuals were developed

		LV			PV		
	df	MS	F	*p*-value	MS	F	*p*-value
(a)							
Population	2	27400	65.26*	<0.001	795	3.9*[a]	0.020
Temperature	1	7191	17.11*	<0.001	22600	112.2*	<0.001
P × T	2	4754	11.31*	<0.001	5109	25.4*	<0.001
Error	210	420			201		
(b)							
Population	2	0.009	3.658*[a]	0.027	0.002	1.5	0.226
Temperature	1	0.002	0.689	0.407	0.038	26.14*	<0.001
P × T	2	0.003	1.316	0.270	0.02	13.73*	<0.001
Error	210	0.002			0.001		

Abbreviations: *df*, degrees of freedom; MS, mean squares.
[a]Not significant after Bonferroni correction for multiple tests ($P_B = 0.01$). $^*p < 0.05$.

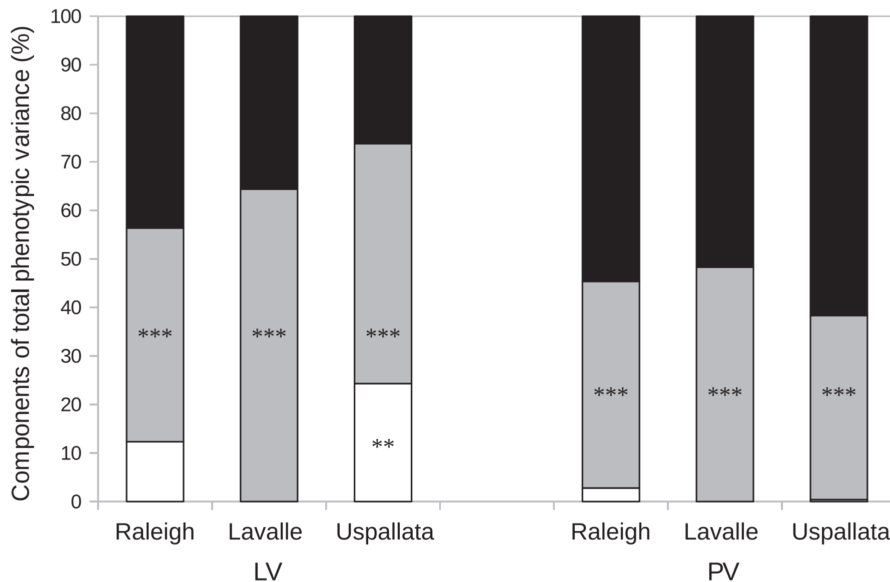

FIGURE 2 Components of total phenotypic variance (in percentage): natural genetic variation (line effect, white block), genotype by environment interaction (line by temperature interaction, grey block) and the error (black block) for larval viability (LV) and pupal viability (PV). The analyses were performed in lines collected in Raleigh, Lavalle and Uspallata that were reared at 17° and 25°C. Significant factors are represented as $^{**}p < 0.01$ and $^{***}p < 0.001$

(Del Pino, Salgado, & Godoy-Herrera, 2012; Horváth & Kalinka, 2016; Satorre, Fanara, & Lavagnino, 2014). Moreover, we found that the genetic basis of each trait varied across the temperatures assessed, as the cross-environment genetic correlations for all traits in the three populations were far from unity. These results suggest that susceptibility to different causes of lethality changes according to rearing temperature, probably in response to the alteration of genetic networks involved in developmental processes, behaviour, immunity and/or resistance to different stressful stimuli. This change in genetic ranking across environments (crossover of reaction norms) would play an important role as contributor to genotype-environment interaction, implying the possibility of independent evolution of these traits at each temperature. A high contribution of changes in ranking order to the significant genotype-environment interaction has also been found for preadult viability in different studies performed with several *Drosophila* species considering diverse environmental factors (Dobzhansky & Spassky, 1944; Fanara et al., 2006; Horváth & Kalinka, 2016; our results). Changes in ranking order imply that no genotypes are associated to phenotypic optima across temperatures; given that natural populations face continuous temperature changes, this fact partly explains the maintenance of a high level of genetic variability associated to the trait.

Although *Drosophila* development encompasses successive developmental stages characterized by different biological processes and behaviours, in most population genetics and evolutionary studies, preadult stages are not differentiated to evaluate potential changes in the genetic basis and factors affecting the evolution of developmental traits. Here, we detected no significant correlations between LV and PV, and found differences in variance components and quantitative genetic parameters for these preadult traits. These results suggest that the genetic basis for viability differs between stages of development, and consequently, both traits (LV and PV) are able to evolve independently. Other studies have shown decoupling

TABLE 4 Estimates of quantitative genetic parameters of larval viability (LV) and pupal viability (PV) in the Raleigh (R), Lavalle (L) and Uspallata (U) populations. H^2 stands for broad sense heritability (estimated with pooled temperatures, at 17°C and 25°C). CV_G and CV_E represent coefficients of genetic and environmental variance, respectively. r_{GxE} is the genetic correlation across environments (17°C and 25°C). GxE_V and GxE_R stand for the decomposition of GxE interaction in variance and genetic ranking changes across environment respectively

	H^2	H^2 17°C	H^2 25°C	CV_G	CV_E	r_{GxE}	$G \times E_V$	$G \times E_R$
LV								
R	0.45	0.45	0.49	16.07	14.14	0.250	7.54	92.46
L	0.48	0.43	0.28	27.75	20.65	−0.159	0.33	99.67
U	0.38	0.51	0.22	27.65	16.51	0.314	1.35	98.65
PV								
R	0.78	0.73	0.82	10.63	11.66	0.078	23.48	76.52
L	0.65	0.63	0.67	14.09	14.58	−0.529	16.21	83.79
U	0.73	0.70	0.80	9.47	12.02	0.003	13.71	86.29

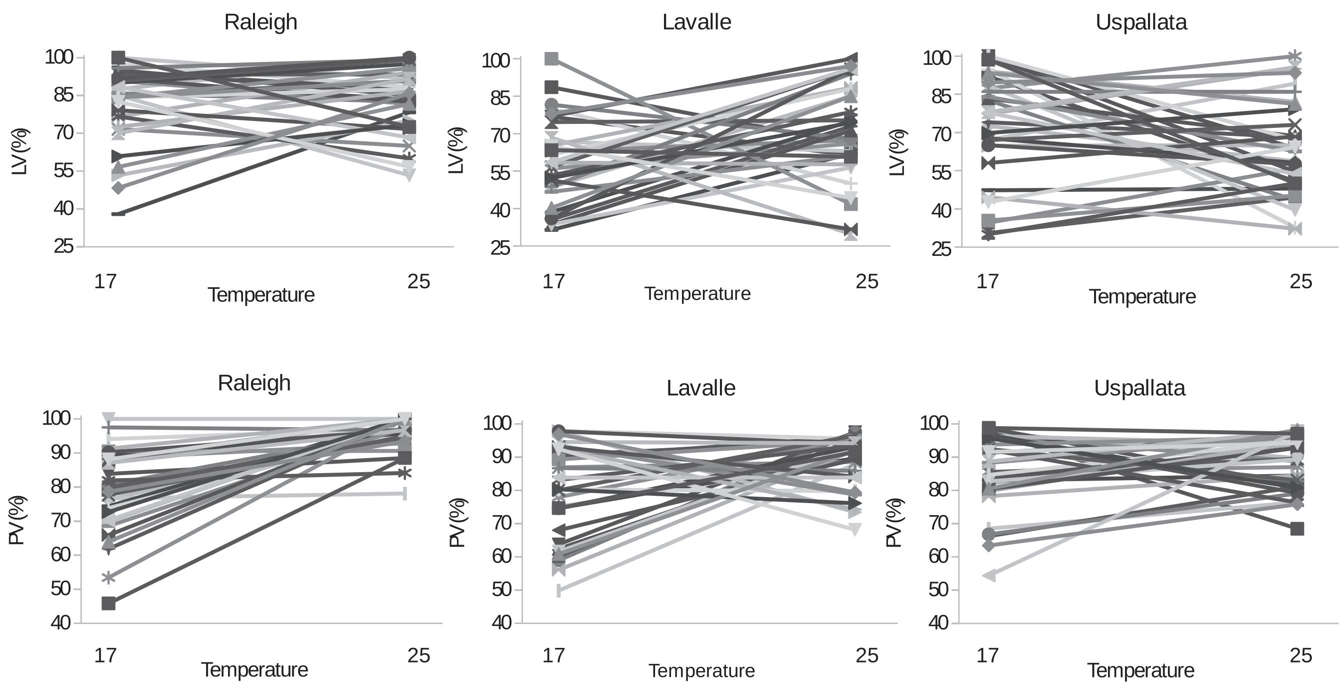

FIGURE 3 Reaction norms for LV (top row) and PV (bottom row) means by line at 17°C and 25°C for the three populations

between larval and pupal traits (Chippindale, Alipaz, & Rose, 2004; Chippindale et al., 1996; Mensch et al., 2010; Partridge, 1992; Partridge, Barrie, Fowler, & French, 1994; Petino Zappala, Ortiz, & Fanara, 2018; Artieri & Singh, 2010), and substantial changes in transcriptional patterns during ontogeny have also been reported (Arbeitman et al., 2002; Gerstein et al., 2014). Moreover, studies on preadult viability performed in other holometabolous insects, when accounting for ontogenetic decoupling, showed similar results concerning the stage and also environmental-specific nature of phenotypic variability (Eskafi & Fernandez, 1990; Liu, Chen, & Zalucki, 2002; Rausher, 1979; Rueda, Patel, Axtell, & Stinner, 1990); nonetheless, the decomposition of traits according to developmental stages is frequently overlooked in studies using *Drosophila*. Accounting for this decoupling is important not only because measuring egg-to-adult viability represents a loss of information, but also because preadult viability condenses the differential susceptibility to a wide range of stressors (including internal, e.g. developmental problems, or external, such as infections) whose incidences vary throughout developmental stages. Furthermore, modularity entails important implications for the maintenance of genetic variability and evolvability, and therefore, it should be addressed (Le Rouzic & Carlborg, 2008; Wagner & Zhang, 2011; Yang, 2001).

We should note that preadult viability is a particularly complex trait affected by disparate factors, and therefore, it could be argued that our results may be not generalizable. However, studies on the genetic architecture of developmental time and pupation height performed using equal thermal treatments on the same *D. melanogaster* lines confirmed their genetic bases are also temperature- and

TABLE 5 Pearson's correlations between means and environmental variation coefficients (CVEs) at each temperature (a) and between plasticities for means and CVEs (b) for larval viability (LV) and pupal viability (PV) among isogenic lines derived from Raleigh (R), Lavalle (L) and Uspallata (U)

	LV		PV	
	17°	25°	17°	25°
(a)				
R	−0.787**	−0.427 (p = 0.012)*[a]	−0.838**	−0.734**
L	−0.308 (p = 0.770)	−0.170 (p = 0.338)	−0.630**	−0.768**
U	−0.430 (p = 0.011)*[a]	−0.139 (p = 0.434)	−0.636**	−0.748**
	LV	**PV**		
(b)				
R	−0.485 (p = 0.002)*	−0.787**		
L	−0.131 (p = 0.461)	−0.722**		
U	−0.089 (p = 0.618)	−0.764**		

[a]Not significant after Bonferroni correction for multiple tests ($P_B = 0.01$). *$p < 0.05$; **$p < 0.01$.

TABLE 6 Components of environmental variation (as defined by Ørsted et al., 2018) for the Raleigh, Uspallata and Lavalle populations for larval viability (LV) and pupal viability (PV). CVE stands for environmental variation coefficient. Δ CVEs correspond to plasticities for CVEs

	Raleigh		Lavalle		Uspallata	
	17°C	25°C	17°C	25°C	17°C	25°C
Mean LV (%)	81.3	83.8	70.4	58.6	74.6	64.5
Mean PV (%)	77.5	94.6	79.3	88.5	86.2	87.8
CVE LV	0.07	0.06	0.08	0.10	0.07	0.07
CVE PV	0.08	0.02	0.07	0.05	0.05	0.05
Plasticity LV (%)	0.97		1.20		1.16	
Plasticity PV (%)	0.82		0.90		0.98	
Δ CVE LV	0.01		−0.02		−0.01	
Δ CVE PV	0.06		0.02		0.00	

stage-specific (Petino Zappala et al., 2018). These results suggest that ontogenetic decoupling and the effect of environmental factors should be accounted for when studying the genetic architecture of other quantitative developmental traits.

Previous studies have also addressed the importance of phenotypic canalization in the maintenance of genetic variability, allowing adaptation to sudden environmental change, promoting invasiveness and overall increasing evolvability (Davidson, Jennions, & Nicotra, 2011; DeWitt, 2016; Flatt, 2005; Pigliucci, 2008), but this aspect of phenotype is also frequently neglected. Here, we detected different patterns of genetic and environmental canalization (i.e. genetic and environmental variance) in the three populations according to rearing temperatures. Generally, temperature affected both components of canalization the same way (i.e. for each population, genetic variance and mean CVE behaved similarly according to the raising temperature). This result is consistent with an interplay between, or a single cause for, both components of canalization (Meiklejohn & Hartl, 2002). For example, under stable environmental conditions, the fixation of canalizing alleles or an accumulation of epistatic interactions "hiding" genetic variability could lower genetic and environmental variance within a population. In case of genetic or environmental perturbations, the alteration of genetic networks could expose previously cryptic genetic variability and also lower environmental robustness. Therefore, our results support the hypothesis that genetic canalization and environmental canalization for preadult viability traits are under genetic control and are probably subject to natural selection.

Whether means and different aspects of environmental variance for different traits share a common genetic basis is a matter of debate. Here, we observed negative correlations between PV means and its CVEs. This finding was expected, since it has been shown that phenotypic decanalization is usually associated to developmental instability and tends to correlate with low viability (Baer, 2008; Bouclier & Biémont, 1982; Møller & Manning, 2003), which could suggest a shared genetic basis for both aspects of phenotype. However, in the case of viability traits, it can be argued that lines with high viability (and therefore near the upper limit of this variable) would necessarily present a lower environmental variance, generating a spurious negative correlation. Furthermore, the correlation is never significant when mean phenotypic values drop below 70%. This suggests that indeed, the correlation between PV means and CVEs would be an artefact caused by its mean values near the variable's upper limit. On the other hand, no conserved correlations were found between LV means and its CVEs. This finding underscores the complex relationships between variation for phenotypic means and environmental canalization for different traits (Blasco et al., 2017; Harbison et al., 2013; Morgante et al., 2015; Reed et al., 2010; Varón-González, Pallares, Debat, & Navarro,

2019). This dependence on the considered trait also holds for the relationships between plasticity and phenotypic variance within environments (Ørsted et al., 2018; Siegal & Leu, 2014; Takahashi, Okada, & Teramura, 2012; Valladares, Balaguer, Martinez-Ferri, Perez-Corona, & Manrique, 2002). That most genetic variants only have an effect restricted to a particular aspect of phenotype (i.e. means or CVEs) would prevent the loss of genetic variability due to selective pressures acting on either aspect. This implication is particularly important for traits with a great impact on fitness, in which the pressure for phenotypic canalization is stronger (Stearns & Kawecki, 1994). All in all, we underscore the need to work on a realistic characterization of genetic architecture of complex traits, including the study of environmental variance components, and assessing the possible modularity in different levels (e.g. genetic, ontogenetic, anatomical), which is essential to accurately describe the robustness and flexibility of the genetic networks maintaining diversity and underlying relevant ecological strategies and evolutionary phenomena.

Further work is needed to confirm by means of Genome-wide Association Studies whether the genetic bases for viability traits are indeed population, stage and temperature-specific, and which biological processes underlie phenotypic variability for these traits.

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DISCLOSURE

The authors declare no conflict of interest.

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REFERENCES

Arbeitman, M. N., Furlong, E. E. M., Imam, F., Johnson, E., Null, B. H., Baker, B. S., … White, K. P. (2002). Gene expression during the life cycle of *Drosophila melanogaster*. *Science*, *297*(5590), 2270–2275.https://doi.org/10.1126/science.1072152

Baer, C. F. (2008). Quantifying the decanalizing effects of mutations in rhabditid nematodes. *The American Naturalist*, *172*, 272–281. https://doi.org/10.1086/589455

Blasco, A., Martínez-Álvaro, M., García, M. L., Ibáñez-Escriche, N., & Argente, M. J. (2017). Selection for environmental variance of litter size in rabbits. *Genetics Selection Evolution*, *49*(1), 48. https://doi.org/10.1186/s12711-017-0323-4

Bolker, J. A. (2000). Modularity in development and why it matters to evo-devo. *American Zoology*, *40*(5), 770–776.

Bouclier, F., & Biémont, C. (1982). Morphological variation and preadult viability at one-generation inbreeding in *Drosophila melanogaster*: A family selection hypothesis. *Genetica*, *60*(1), 13–17. https://doi.org/10.1007/BF00121450

Casares, P., & Carracedo, M. C. (1987). Pupation height in *Drosophila*: Sex differences and influence of larval developmental time. *Behavior Genetics*, *17*(5), 523–535. https://doi.org/10.1007/BF01073119

Chippindale, A. K., Alipaz, J. A., & Rose, M. R. (2004). Experimental evolution of accelerated development in Drosophila. 2. Adult fitness and the fast development syndrome. In M. R. Rose, H. B. Passananti and M. Matos (Eds.), *Methuselah Flies: A Case Study in the Evolution of Aging*, Singapore: World Scientific Publishing.

Chippindale, A. K., Chu, T. J. F., & Rose, M. R. (1996). Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution*, *50*(2), 753–766. https://doi.org/10.1111/j.1558-5646.1996.tb03885.x

Davidson, A. M., Jennions, M., & Nicotra, A. B. (2011). Do invasive species show higher phenotypic plasticity than native species and if so, is it adaptive? A meta-analysis *Ecology Letters*, *14*(4), 419–431. https://doi.org/10.1111/j.1461-0248.2011.01596.x

Debat, V., & Le Rouzic, A. (2019). Canalization, a central concept in biology, *Seminars in cell & developmental biology*, *88*, 1–3. https://doi.org/10.1016/j.semcdb.2018.05.012

Del Pino, F., Salgado, E., & Godoy-Herrera, R. (2012). Plasticity and genotype - environment interactions for locomotion in *Drosophila melanogaster* larvae. *Behavior Genetics*, *42*(1), 162–169. https://doi.org/10.1007/s10519-011-9490-1

DeWitt, T. J. (2016). Expanding the phenotypic plasticity paradigm to broader views of trait space and ecological function. *Current Zoology*, *62*(5), 463–473. https://doi.org/10.1093/cz/zow085

DeWitt, T. J., & Scheiner, S. M. (2004). *Phenotypic plasticity: functional and conceptual approaches*. New York, NY: Oxford University Press.

Dobzhansky, T., & Spassky, B. (1944). Genetics of natural populations. XI. Manifestation of genetic variants in *Drosophila pseudoobscura* in different environments. *Genetics*, *29*(3), 270.

Eskafi, F. M., & Fernandez, A. (1990). Larval–pupal mortality of Mediterranean fruit fly (Diptera: Tephritidae) from interaction of soil, moisture, and temperature. *Environmental Entomology*, *19*(6), 1666–1670. https://doi.org/10.1093/ee/19.6.1666

Falconer, D. S., & Mackay, T. F. C. (1996) *Introduction to quantitative genetics*. Essex, UK: Longman.

Fanara, J. J., Folguera, G., Fernandez Iriarte, P., Mensch, J., & Hasson, E. (2006). Genotype by environment interactions in viability and developmental time in populations of cactophilic *Drosophila*. *Journal of Evolutionary Biology*, *19*, 900–906. https://doi.org/10.1111/j.1420-9101.2006.01084.x

Flatt, T. (2005). The evolutionary genetics of canalization. *The Quarterly Review of Biology*, *80*(3), 287–316. https://doi.org/10.1086/432265

Folguera, G., Ceballos, S., Spezzi, L., Fanara, J. J., & Hasson, E. (2008). Clinal variation in developmental time and viability, and the response to thermal treatments in two species of *Drosophila*. *Biological Journal of the Linnean Society*, *95*(2), 233–245. https://doi.org/10.1111/j.1095-8312.2008.01053.x

Fowler, K., Semple, C., Barton, N. H., & Partridge, L. (1997). Genetic variation for total fitness in *Drosophila melanogaster*. *Proceedings of the Royal Society*, *264*, 191–199. https://doi.org/10.1098/rspb.1997.0027

Futuyma, D. J. (1998). *Evolutionary biology* (3rd ed.). Sunderland, MA: Sinauer Associates, Inc.

Gardner, M., Fowler, K., Partridge, L., & Barton, N. (2001). Genetic variation for preadult viability in *Drosophila melanogaster*. *Evolution*, *55*(8), 1609–1620. https://doi.org/10.1111/j.0014-3820.2001.tb00680.x

Gerstein, M. B., Rozowsky, J., Yan, K. K., Wang, D., Cheng, C., Brown, J. B., … Waterston, R. (2014). Comparative analysis of the transcriptome across distant species. *Nature*, *512*(7515), 445–448. https://doi.org/10.1038/nature13424

Gilchrist, G. W., & Lee, C. E. (2007). All stressed out and nowhere to go: Does evolvability limit adaptation in invasive species? An

introduction to the symposium at the SSE/ASN/SSB meeting, June 2004. *Genetica*, *129*(2), 127–132. https://doi.org/10.1007/s10709-006-9009-5

Goldstein, D. B., & Pollock, D. D. (1997). Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. *Journal of Heredity*, *88*(5), 335–342. https://doi.org/10.1093/oxfordjournals.jhered.a023114

Hansen, T. F. (2003). Is modularity necessary for evolvability?: Remarks on the relationship between pleiotropy and evolvability. *Biosystems*, *69*(2–3), 83–94. https://doi.org/10.1016/S0303-2647(02)00132-6

Hansen, T. F. (2006). The evolution of genetic architecture. *Annual Review of Ecology Evolution and Systematics*, *37*(1), 123–157. https://doi.org/10.1146/annurev.ecolsys.37.091305.110224

Harbison, S. T., McCoy, L. J., & Mackay, T. F. C. (2013). Genome-wide association study of sleep in *Drosophila melanogaster*. *BMC Genomics*, *14*(1), 281. https://doi.org/10.1186/1471-2164-14-281

Hill, W. G., & Zhang, X. S. (2012). On the pleiotropic structure of the genotype-phenotype map and the evolvability of complex organisms. *Genetics*, *190*, 1131–1137. https://doi.org/10.1534/genetics.111.135681

Hoekstra, H. E., & Coyne, J. A. (2007). The locus of evolution: Evo devo and the genetics of adaptation. *Evolution*, *61*(5), 995–1016. https://doi.org/10.1111/j.1558-5646.2007.00105.x

Horváth, B., & Kalinka, A. T. (2016). Effects of larval crowding on quantitative variation for development time and viability in *Drosophila melanogaster*. *Ecology and Evolution*, *6*(23), 8460–8473. https://doi.org/10.1002/ece3.2552

Hughes, K. A., & Leips, J. (2017). Pleiotropy, constraint, and modularity in the evolution of life histories: Insights from genomic analyses. *Annals of the New York Academy of Sciences*, *1389*, 76–91. https://doi.org/10.1111/nyas.13256

Lavagnino, N. J., Anholt, R. R. H., & Fanara, J. J. (2008). Variation in genetic architecture of olfactory behaviour among wild-derived populations of *Drosophila melanogaster*. *Journal of Evolutionary Biology*, *21*(4), 988–996. https://doi.org/10.1111/j.1420-9101.2008.01546.x

Le Rouzic, A., & Carlborg, Ö. (2008). Evolutionary potential of hidden genetic variation. *Trends in Ecology & Evolution*, *23*(1), 33–37. https://doi.org/10.1016/j.tree.2007.09.014

Liu, S. S., Chen, F. Z., & Zalucki, M. P. (2002). Development and survival of the diamondback moth (Lepidoptera: Plutellidae) at constant and alternating temperatures. *Environmental Entomology*, *31*(2), 221–231. https://doi.org/10.1603/0046-225X-31.2.221

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits* (Vol. *1*, pp. 535–557). Sunderland, MA: Sinauer.

Mackay, T. F. C. (1986). A quantitative genetic analysis of fitness and its components in *Drosophila melanogaster*. *Genetical Research*, *47*, 59–70. https://doi.org/10.1017/S0016672300024526

Mackay, T. F. C. (2001). The genetic architecture of quantitative traits. *Annual Review of Genetics*, *35*(1), 303–339. https://doi.org/10.1146/annurev.genet.35.102401.090633

Mackay, T. F. C., & Huang, W. (2018). Charting the genotype–phenotype map: lessons from the *Drosophila melanogaster* Genetic Reference Panel. *Wiley Interdisciplinary Reviews-Developmental Biology*, *7*(1), e289. https://doi.org/10.1002/wdev.289

Mackay, T. F. C., Richards, S., Stone, E. A., Barbadilla, A., Ayroles, J. F., Zhu, D., … Gibbs, R. A. (2012). The *Drosophila melanogaster* genetic reference panel. *Nature*, *482*(7384), 173–178. https://doi.org/10.1038/nature10811

Meiklejohn, C. D., & Hartl, D. L. (2002). A single mode of canalization. *Trends in Ecology & Evolution*, *17*(10), 468–473. https://doi.org/10.1016/S0169-5347(02)02596-X

Mensch, J., Carreira, V., Lavagnino, N., Goenaga, J., Folguera, G., Hasson, E., … Fanara, J. J. (2010). Stage-specific effects of candidate heterochronic genes on variation in developmental time along an altitudinal cline of *Drosophila melanogaster*. *PLoS ONE*, *5*(6), e11229. https://doi.org/10.1371/journal.pone.0011229

Minelli, A., Brena, C., Deflorian, G., Maruzzo, D., & Fusco, G. (2006). From embryo to adult—beyond the conventional periodization of arthropod development. *Development Genes and Evolution*, *216*(7–8), 373–383. https://doi.org/10.1007/s00427-006-0075-6

Møller, A. P., & Manning, J. (2003). Growth and developmental instability. *Journal of Veterinary Science*, *166*(1), 19–27.

Morgante, F., Sørensen, P., Sorensen, D. A., Maltecca, C., & Mackay, T. F. C. (2015). Genetic architecture of micro-environmental plasticity in *Drosophila melanogaster*. *Scientific Reports*, *5*, 9785. https://doi.org/10.1038/srep09785

Mousseau, T. A., & Roff, D. A. (1987). Natural selection and the heritability of fitness components. *Heredity*, *59*, 181–197. https://doi.org/10.1038/hdy.1987.113

Muir, W., Nyquist, W. E., & Xu, S. (1992). Alternative partitioning of the genotype-by-environment interaction. *TAG. Theoretical and Applied Genetics.*, *84*(1–2), 193–200. https://doi.org/10.1007/BF00224000

van Noordwijk, A. J., & de Jong, G. (1986). Acquisition and allocation of resources: Their influence on variation in life history tactics. *The American Naturalist*, *128*(1), 137. https://doi.org/10.1086/284547

Ørsted, M., Rohde, P. D., Hoffmann, A. A., Sørensen, P., & Kristensen, T. N. (2018). Environmental variation partitioned into separate heritable components. *Evolution*, *72*(1), 136–152. https://doi.org/10.1111/evo.13391

Paaby, A., & Gibson, G. (2016). Cryptic genetic variation in evolutionary developmental genetics. *Biology (Basel)*, *5*(2), 28.

Paaby, A.s & Testa, N. D. (2018). Developmental plasticity and evolution. In L. Nuno de la Rosa & G. Müller (Eds.), *Evolutionary Developmental Biology* (pp. 1-14). Cham: Springer.

Partridge, L. (1992). Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Evolution*, *46*(1), 76–91. https://doi.org/10.1111/j.1558-5646.1992.tb01986.x

Partridge, L., Barrie, B., Fowler, K., & French, V. (1994). Thermal evolution of pre-adult life history traits in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, *7*(6), 645–663. https://doi.org/10.1046/j.1420-9101.1994.7060645.x

Petino Zappala, M. A., Ortiz, V. E., & Fanara, J. J. (2018). Study of natural genetic variation in early fitness traits reveals decoupling between larval and pupal developmental time in *Drosophila melanogaster*. *Evolutionary Biology*, *45*(4), 437–448, 1–12.

Pigliucci, M. (2008). Is evolvability evolvable? *Nature Reviews Genetics*, *9*(1), 75–82. https://doi.org/10.1038/nrg2278

Prasad, N. G., Shakarad, M., Anitha, D., Rajamani, M., & Joshi, A. (2001). Correlated responses to selection for faster development and early reproduction in *Drosophila*: the evolution of larval traits. *Evolution*, *55*(7), 1363–1372. https://doi.org/10.1111/j.0014-3820.2001.tb00658.x

Prasad, N. G., Shakarad, M., Gohil, V. M., Sheeba, V., Rajamani, M., & Joshi, A. (2000). Evolution of reduced pre-adult viability and larval growth rate in laboratory populations of *Drosophila melanogaster* selected for shorter development time. *Genetical Research*, *76*(3), 249–259. https://doi.org/10.1017/S0016672300004754

Artieri, C. G., & Singh, R. S. (2010). Molecular evidence for increased regulatory conservation during metamorphosis, and against deleterious cascading effects of hybrid breakdown in Drosophila. *BMC biology*, *8*(1), 29.

Rausher, M. D. (1979). Larval habitat suitability and oviposition preference in three related butterflies. *Ecology*, *60*(3), 503–511. https://doi.org/10.2307/1936070

Reed, L. K., Williams, S., Springston, M., Brown, J., Freeman, K., DesRoches, C. E., … Gibson, G. (2010). Genotype-by-diet interactions drive metabolic phenotype variation in *Drosophila melanogaster*. *Genetics*, *185*(3), 1009–1019. https://doi.org/10.1534/genetics.109.113571

Robertson, A. (1959). The sampling variance of the genetic correlation coefficient. *Biometrics*, *15*(3), 469–485. https://doi.org/10.2307/2527750

Rodríguez-Ramilo, S. T., Pérez-Figueroa, A., Fernández, B., Fernández, J., & Caballero, A. (2004). Mutation-selection balance accounting for genetic variation for viability in *Drosophila melanogaster* as deduced from an inbreeding and artificial selection experiment. *Journal of Evolutionary Biology*, *17*(3), 528–541. https://doi.org/10.1111/j.1420-9101.2004.00707.x

Rueda, L. M., Patel, K. J., Axtell, R. C., & Stinner, R. E. (1990). Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, *27*(5), 892–898. https://doi.org/10.1093/jmedent/27.5.892

Satorre, I., Fanara, J. J., & Lavagnino, N. (2014). Micro-geographical scale variation in *Drosophila melanogaster* larval olfactory behaviour is associated with host fruits heterogeneity. *Entomologia Experimentalis et Applicata*, *152*, 23–30. https://doi.org/10.1111/eea.12192

Siegal, M. L., & Leu, J. Y. (2014). On the nature and evolutionary impact of phenotypic robustness mechanisms. *Annual Review of Ecology Evolution and Systematics*, *45*, 495–517. https://doi.org/10.1146/annurev-ecolsys-120213-091705

Sørensen, P., de los Campos, G., Morgante, F., Mackay, T. F., & Sorensen, D. (2015). Genetic control of environmental variation of two quantitative traits of *Drosophila melanogaster* revealed by whole-genome sequencing. *Genetics*, *201*, 487–497. https://doi.org/10.1534/genetics.115.180273

Stearns, S. C. (1992). *The evolution of life histories*. Oxford, UK: Oxford University Press.

Stearns, S. C., & Kawecki, T. J. (1994). Fitness sensitivity and the canalization of life-history traits. *Evolution*, *48*(5), 1438–1450. https://doi.org/10.1111/j.1558-5646.1994.tb02186.x

Takahashi, K. H., Okada, Y., & Teramura, K. (2012). Deficiency screening for genomic regions with effects on environmental sensitivity of the sensory bristles of *Drosophila melanogaster*. *Evolution*, *66*(9), 2878–2890. https://doi.org/10.1111/j.1558-5646.2012.01636.x

Valladares, F., Balaguer, L., Martinez-Ferri, E., Perez-Corona, E., & Manrique, E. (2002). Plasticity, instability and canalization: is the phenotypic variation in seedlings of sclerophyll oaks consistent with the environmental unpredictability of Mediterranean ecosystems? *New Phytologist*, *156*(3), 457–467. https://doi.org/10.1046/j.1469-8137.2002.00525.x

Varón-González, C., Pallares, L. F., Debat, V., & Navarro, N. (2019). Mouse skull mean shape and shape robustness rely on different genetic architectures and different loci. *Frontiers in Genetics*, *10*, 64. https://doi.org/10.3389/fgene.2019.00064

Wagner, G. P., & Zhang, J. (2011). The pleiotropic structure of the genotype–phenotype map: The evolvability of complex organisms. *Nature Reviews Genetics*, *12*(3), 204–213. https://doi.org/10.1038/nrg2949

Yang, A. S. (2001). Modularity, evolvability, and adaptive radiations: A comparison of the hemi- and holometabolous insects. *Evolution & Development*, *3*(2), 59–72. https://doi.org/10.1046/j.1525-142x.2001.003002059.x

SUPPORTING INFORMATION

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