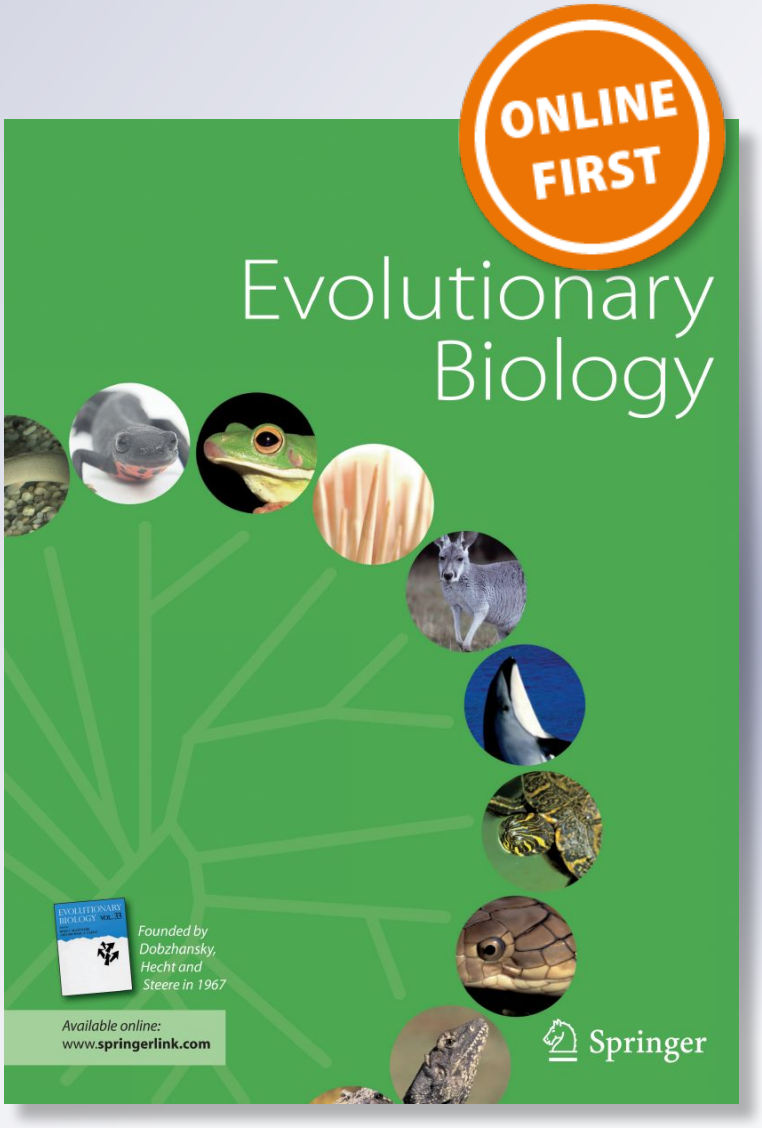
Study of Natural Genetic Variation in Early Fitness Traits Reveals Decoupling Between Larval and Pupal Developmental Time in Drosophila melanogaster

M. A. Petino Zappala, V. E. Ortiz & J. J. Fanara

Evolutionary Biology
Evolutionary Biology

ISSN 0071-3260

Evol Biol
DOI 10.1007/s11692-018-9461-z



Springer

Evolutionary Biology
https://doi.org/10.1007/s11692-018-9461-z

RESEARCH ARTICLE

Study of Natural Genetic Variation in Early Fitness Traits Reveals Decoupling Between Larval and Pupal Developmental Time in *Drosophila melanogaster*

M. A. Petino Zappala[1] · V. E. Ortiz[1] · J. J. Fanara[1]

Received: 12 April 2018 / Accepted: 8 October 2018

Abstract
Characterizing the relationships between genotype and phenotype for developmental adaptive traits is essential to understand the evolutionary dynamics underlying biodiversity. In holometabolous insects, the time to reach the reproductive stage and pupation site preference are two such traits. Here we characterize aspects of the genetic architecture for Developmental Time (decomposed in Larval and Pupal components) and Pupation Height using lines derived from three natural populations of *Drosophila melanogaster* raised at two temperatures. For all traits, phenotypic differences and variation in plasticity between populations suggest adaptation to the original thermal regimes. However, high variability within populations shows that selection does not exhaust genetic variance for these traits. This could be partly explained by local adaptation, environmental heterogeneity and modifications in the genetic architecture of traits according to environment and ontogenetic stage. Indeed, our results show that the genetic factors affecting Developmental Time and Pupation Height are temperature-specific. Varying relationships between Larval and Pupal Developmental Time between and within populations also suggest stage-specific modifications of genetic architecture for this trait. This flexibility would allow for a somewhat independent evolution of adaptive traits at different environments and life stages, favoring the maintenance of genetic variability and thus sustaining the traits' evolvabilities.

Keywords Developmental time · Pupation height · Genetic variation · Phenotypic plasticity · Ontogenetic decoupling

Introduction

The study of Life History Traits allows to link phenotypic variability and different ecological adaptive strategies through changes in the acquisition and allocation of resources (Flatt and Heyland 2011; van Noordwijk and de Jong 1986; Roff 1992; Stearns 1989, 1992).

The time elapsed from the embryogenesis to the reproductive phase is one such trait, commonly known as Developmental Time; in holometabolous insects, it can be conceived as the sum of the duration of successive discrete phases: embryonic, larval and pupal stages, ending with the emergence of the imago. In organisms that develop in ephemeral substrates, such as *Drosophila melanogaster*, a faster development is fundamental for higher pre-adult survival (Partridge and Fowler 1992; Nylin and Gotthard 1998; Narasimha et al. 2015). However, it has been demonstrated that an acceleration of development determines a reduction of fitness related to different adult traits (Partridge and Fowler 1992; Nunney 1996, 2007; Bharathi et al. 2004; Kingsolver and Huey 2008). This pattern, called fast development syndrome, involves trade-offs or negative correlations between developmental rate and body growth (Chippindale et al. 2004). Variations in growth patterns, growth rate and the duration of the growth period affect the age and size at maturity, implying that the study of juvenile growth may be crucial to understand life history evolution since smaller body size has a negative impact on stress resistance (Matzkin et al. 2007; Gilchrist et al. 2008), immune response (Jumbo-Lucioni et al. 2010) and fertility (Nunney 1996; Long et al. 2009). In holometabolous insects, body size and development time are controlled by three factors: (i) growth rate, (ii) the time required to attain a critical size and (iii) the

✉ J. J. Fanara
jjfanara@ege.fcen.uba.ar

[1] Departamento de Ecologia, Genetica y Evolucion - IEGEBA (CONICET-UBA), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires Ciudad Universitaria, Pabellon II, 1428 Buenos Aires, Argentina

Published online: 12 October 2018

interval to cessation of growth that corresponds to the onset of pupation and metamorphosis (Davidowitz and Nijhout 2004). Early in the last larval stage, larvae reach a critical size, at which point they have acquired enough nutrients to complete development, stop feeding and search for a pupation site. These biological processes depend on a genetic program regulated by different hormone pulses associated to each ontogenetic stage; their timing displays considerable natural genetic variation (Fanara et al. 2006; Mensch et al. 2010) and is also affected by environmental conditions, enabling it to adapt to different ecological scenarios (Prasad et al. 2001; Paranjpe et al. 2005; Shingleton et al. 2005; Edgar 2006; Mirth and Riddiford 2007; Mensch et al. 2008; Werenkraut et al. 2008; Rewitz et al. 2013; Rodrigues et al. 2015). Therefore, the timing of insect metamorphosis is not a simple function of chronological age.

The traditional framework for the description of *Drosophila* development takes the molt-to-molt interval as the fundamental unit of periodization, which is similar to the morphological picture of the main body axis as a series of segments (Minelli et al. 2006). Considering that both larval and pupal ontogenetic stages have anatomically, physiologically and behaviorally dissimilar characteristics, they could be analyzed as distinct modules. Indeed, it has been proposed that the modularity between life stages in holometabolous insects results in a greater intra and interspecific diversity (Yang 2001; Minelli et al. 2006). However, Developmental Time is usually not separated into their stages components, but instead studied as a whole. In this sense, decomposing Developmental Time in its larval and pupal parts allows for detection of phenotypic variability that could be compensated if considering total Developmental Time by opposing changes in both components (Nunney 2007). Moreover, the analysis of natural variation for Developmental Time and the study of the relative duration of ontogenetic stages within and between populations can provide relevant information on the mechanisms and processes underlying phenotypic evolution in early Life History Traits.

Pupation site preference is also an important factor in *Drosophila* preadult development because the place selected by larvae to form the puparium can have a decisive influence on viability (Prasad et al. 2001; Beltramí et al. 2010; Del Pino et al. 2014). Since the total fitness is heavily influenced by the larval stages, the pupation site preference constitutes an important component of fitness (Markow 1979). The larvae pupating on the sides of vials close to the food surface are likely to be dislodged and drowned by the larvae crawling on the sides and any larva pupating on the surface of medium is likely to be buried. Pupation Height also enables to estimate pupation site preference (Singh and Pandey 1993) and is tightly linked to resource allocation and acquisition in larval stages (Narasimha et al. 2015) serving as an estimation of motility through the latter larval stage (L3, Riedl et al. 2007). Likewise, Pupation Height plays an important role in niche differentiation between and within species (Welbergen and Sokolowski 1994; Del Pino et al. 2012). This trait harbors substantial genetic variation (Markow 1979; Sokolowski and Hansell 1983; Bauer and Sokolowski 1985; Mueller and Sweet 1986; Singh and Pandey 1993; Riedl et al. 2007) and depends on the sensing of both internal and external cues, i.e. nutritional status, larval crowding, pH, luminosity (Dillon et al. 2009; Del Pino et al. 2012, 2014). In addition, relationships between Pupation Height or the distance covered by wandering Larvae 3 and Larval Developmental Time were found in several studies, mostly in lines artificially selected for fast development (Casares and Carracedo 1987; Chippindale et al. 1997; Prasad et al. 2001; Narasimha et al. 2015). Whether a trade-off between Pupation Height and Larval (and/or Pupal) Developmental Time exists and is maintained in different natural populations and across environments is unknown.

Studying the genetic bases of these developmental adaptive traits involves also an assessment of the effects of environmental variation on the phenotype. Phenotypic plasticity, the property of a genotype to produce different phenotypes in response to varying environments (Schlichting and Pigliucci 1998; Conner and Hartl 2004) is expected to develop in natural populations for traits that present different phenotypic optima across frequently experienced environments (DeWitt and Scheiner 2004; Flatt 2005). Several studies have shown that the evaluation of phenotypic plasticity in life history traits may be important to understand the underlying genetic changes that determine the population dynamics of cosmopolitan species, the ecological fate of genotypes (Carreira et al. 2013; Fallis et al. 2014), and the expansion of species (Fanara and Hasson 2001). In particular, adaptation to different regimes of daily and annually fluctuating temperatures may involve changes in phenotypic plasticity patterns for developmental traits (Angilletta et al. 2004; Davidowitz and Nijhout 2004; Trotta et al. 2006).

In order to characterize the evolution of several aspects of genetic architecture for the aforementioned developmental traits, we measured phenotypic variability for Larval and Pupal Developmental Time and Pupation Height in isogenic lines derived from three natural populations of *Drosophila melanogaster* reared at two different temperatures (17 or 25 °C) and tested if: (i) phenotypic variability between and within populations exists for all traits and it is due to genetic and environmental factors, (ii) patterns of phenotypic plasticity differ between populations, (iii) Larval and Pupal Developmental Time are phenotypically and genetically decoupled, (iv) there are no conserved correlations between characters across populations and temperatures. Our results show significant genetic variation underlying phenotypic differences at both temperatures in all traits analyzed and variation in thermal plasticity between and within

populations. However, adaptive variation in mean thermal plasticity among populations was detected only for Larval Developmental Time. No conserved correlations across all population-temperature combinations were found between traits. Moreover, the genetic bases of Larval and Pupal Developmental Time also differ between populations; the ratio between both traits exhibited significant variation both between and within populations, suggesting that they are decoupled. In the light of these results, we emphasize the importance of studying separately the different aspects of genetic architecture of developmental traits for each ontogenetic stage to elucidate the genetic underpinning that affects natural variation and environmental influence in larval and pupal development.

Materials and Methods

Populations

Three different populations of *Drosophila melanogaster* were analyzed: Raleigh (North Carolina, USA) and two populations collected in Argentina, Lavalle and Uspallata (Province of Mendoza) that are ~70 km away each other (Table 1). Grapevine, plum, cantaloupe and peach were grown in the region of the Lavalle collection site, while Uspallata lines were collected at an apple, pear and quince plantation. *D. simulans* individuals were also collected in both locations, while *D. buzzatii* specimens were only found in Lavalle. The 40 lines from Raleigh used in this study correspond to a subset of the *Drosophila Reference Genome Panel* (DGRP; Mackay et al. 2012; Huang et al. 2014) while 34 lines from each Argentinean population were obtained following the same protocol performed by Mackay et al. (2012) and Huang et al. (2014). Briefly, we established isofemale lines from gravid females collected in Uspallata and Lavalle, and inbred them by 20 generations of full-sib mating, followed by random mating (Mackay et al. 2012). This protocol determined lines with an inbreeding coefficient of 0.986 (Falconer and Mackay 1996). All lines were maintained independently, in cornmeal-agar-molasses medium in an incubator at 25 ± 0.5 °C, under a 12-h light : 12-h dark cycle and at 70% humidity.

Microsatellite analyses (Goldstein and 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 and Satorre, personal communication).

Developmental Time and Pupation Height Assays

For each line, 200 pairs of sexually mature flies were placed in egg collecting chambers with Petri dishes containing an egg-laying medium. Twelve hours later, Petri dishes were removed and eggs were allowed to hatch. Batches of 30 first-instar larvae were transferred to culture vials containing 5 ml of cornmeal-agar-molasses medium. In all three populations, 4–5 replicate vials per combination of line and temperature were set up. Larvae were raised until adult emergence at two controlled temperatures: 17 and 25 °C (±0.5 °C), respectively representing the mean temperatures for summer months in the highland and lowland locations considered (Table 1). In both thermal treatments, the humidity (60–70%) and photoperiod (12:12 light:dark) were equal. Every 24 h, new pupae were counted and emerged flies were collected.

We quantified Larval Developmental Time as the time elapsed since the estimated egg-laying time (at half the oviposition interval) until pupation, by counting the number of new pupae every 24 h. Pupal Developmental Time was calculated as the time elapsed from pupation to emergence of the imago, by counting the number of adult flies emerging from the puparium every 24 h. Pupation height was measured with a caliper as the distance between the surface of the medium and the center of each pupa. For all traits analyzed, replicates were considered as the experimental units.

Differences between populations were evaluated for each trait by means of fixed model analysis of variance (ANOVAs) according to the model

$$y = \mu + P + T + P \times T + \epsilon,$$

where P and T are the fixed effects of Population and Temperature and Ɛ stands for the error. The population effect

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)

Population	Location	Altitude	Annual temperature		Daily thermal amplitude	Annual precipitations
			Average	Min–max		
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

Altitude is given in meters; all temperature information is in °C, and precipitation is expressed in mm. Minimal and maximal temperatures represent the lowest and highest monthly averages

tests for phenotypic differentiation among populations while the P x T stands for variation in thermal plasticity among populations.

To further dissect the natural genetic variation within population, we also performed mixed model ANOVAs for each population separately, based on the model

$$y = \mu + L + T + L \times T + \epsilon,$$

where L is a random effect of Line. In these ANOVAs, significant L and L × T effects are interpreted as the presence of genetic variation and genetic variation in thermal plasticity, respectively. Partial ANOVAs were also performed for each temperature separately.

We estimated quantitative genetic parameters for each natural population. Under our experimental design, genetic (σ_G^2), environmental (σ_E^2) and phenotypic (σ_P^2) variance were estimated as $\sigma_G^2 = \sigma_L^2 + \sigma_{LT}^2$; $\sigma_E^2 = \sigma_W^2$ and $\sigma_P^2 = \sigma_G^2 + \sigma_E^2$, where σ_L^2, σ_{LT}^2, and σ_W^2 are among line, line by temperature and within line variance components, respectively. Broad sense heritability (H^2) for each trait was estimated as $H^2 = \sigma_G^2/\sigma_P^2$. Coefficients of genetic ($CV_G = 100\sigma_G/$mean) and environmental ($CV_E = 100\sigma_E/$mean) variance were also computed. The cross environment (temperature) genetic correlations ($rGxE_{(17-25)}$) is the genetic correlation of measurements of the same trait in different environments (temperatures), and here it reflects the degree in which the same genotype controls trait expression across developmental temperatures. We calculated $rGxE_{(17-25)}$ as $COV_{17-25}/(\sigma_{17}\,\sigma_{25})$, where COV_{17-25} represents the covariance of lines means measured at 17 and 25 °C while σ_{17} and σ_{25} are the square roots of the among lines variance components obtained from the one-way ANOVAs performed for each populations wherein the lines were developed at 17 and 25 °C, respectively. This method for the estimation of $rGxE_{(17-25)}$ is not equivalent to the computation of a product-moment correlation (see Lynch and Walsh 1998, pp. 663). 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 and 25 °C, separately. Correlation analyses were carried out within each temperature for each population. Finally, we computed the ratio Larval to Pupal Developmental Time as an estimation of decoupling between these two heterochronic traits. This ratio was indexed as (ln Larval Developmental Time—ln Pupal Developmental Time), hereafter referred to as Larval DT/Pupal DT, to reduce statistical problems arising from ratio measurements (Atchley et al. 1976).

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

Results

A total of 40 lines from Raleigh, 34 lines from Lavalle and 34 lines from Uspallata were successfully quantified for all traits at both temperatures. We included in the analysis those replicates that had a total viability higher than 25%. Our analysis revealed significant variation among populations and thermal plasticity for both Developmental Time stages and Pupation Height (Table 2). In all cases, highest scores always were quantified at 17 °C, although the patterns of variation for thermal plasticity detected among populations for Larval and Pupal Developmental Time were different, i.e. reaction norms were not parallel between populations. This suggests that the differences between populations depended on the character-temperature combination (Fig. 1). Moreover, populations showed less phenotypic differences at 25 °C than at 17 °C for Developmental Time traits. On the other hand, the significant population effect observed in Pupation Height was attributable to differences exhibited between Raleigh and Lavalle populations, considering that for this trait we did not detect significant population by temperature interaction (Fig. 1; Table 2).

To further investigate the natural genetic variation for each trait we performed ANOVAs within each population. Our results revealed significant genetic variation for all combination of populations and early fitness traits analyzed, being Lavalle the population exhibiting the highest genetic

Table 2 ANOVAs for larval developmental time (LDT), pupal developmental time (PDT) and pupation height (PH) among populations (P), and between temperatures (T) wherein individuals were developed

	LDT		PDT		PH	
	MS	F	MS	F	MS	F
Population	15,875	13.75***	1904	4.61*†	145.43	5.68**
Temperature	1,440,325	1265.81***	735,648	1781.39***	217.480	8.50
P × T	19,369	17.02***	1097	2.66*†	47.60	1.86
Error	1170		413		25.60	

Degrees of freedom: 2, 1, 2, 210 for P, T, P × T and error, respectively. *MS* mean squares

*p < 0.05; **p < 0.01; ***p < 0.001

†Not significant after Bonferroni correction for multiple tests ($P_B = 0.01$)

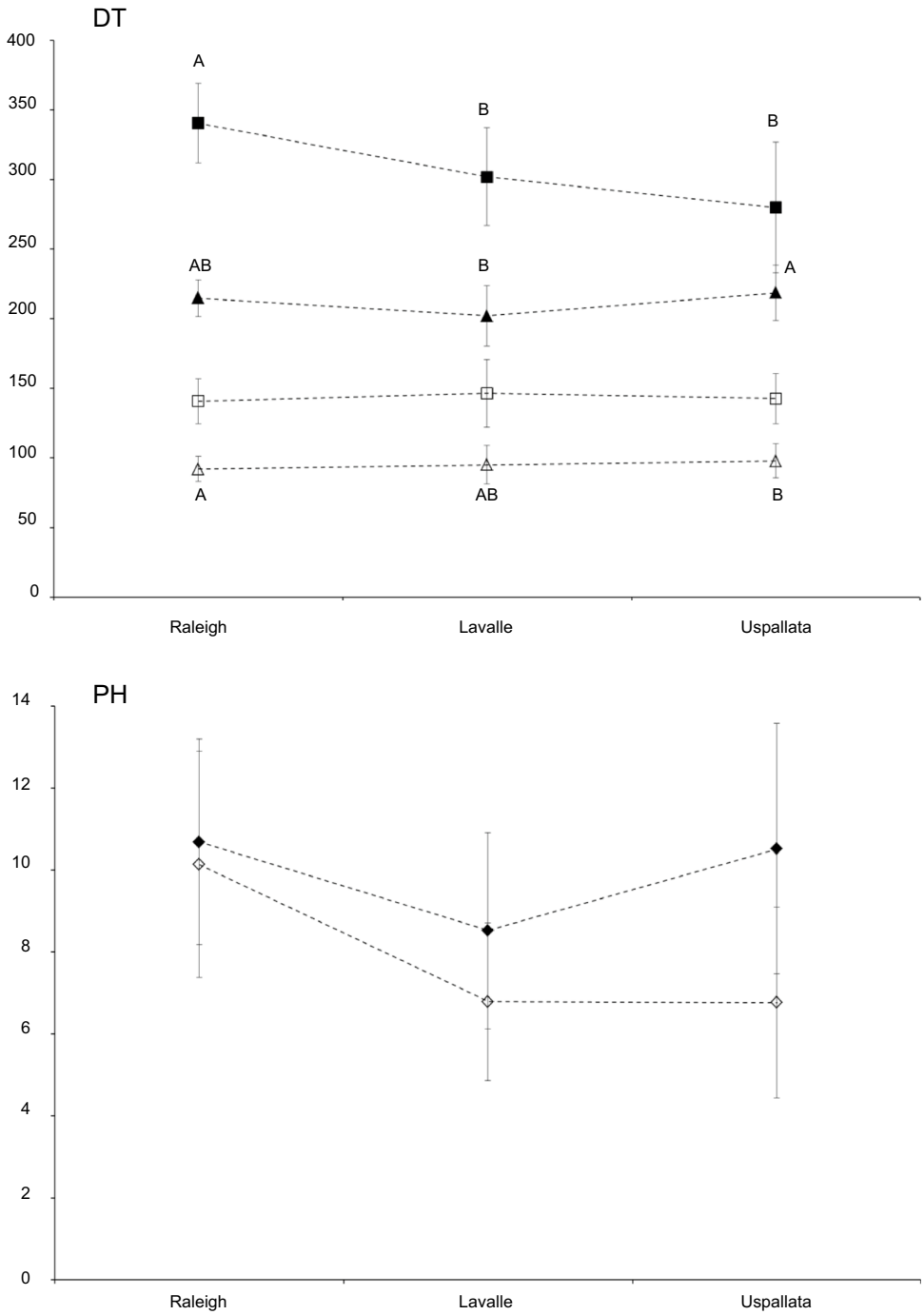


Fig. 1 Mean of Larval Developmental Time (LDT, square), Pupal Developmental Time (PDT, triangle) and Pupation Height (PH) in individuals reared at 17 °C (filled symbol) and 25 °C (open symbol) from Raleigh, Lavalle and Uspallata. LDT and PDT are expressed in hours and PH in mm. Different letters represent significant differences between population according to Tukey´s a posteriori comparison of means ($p < 0.05$). Vertical bars represent standard deviation

variation on average (Fig. 2). It is interesting to note that none of the lines analyzed showed consistent high or low values for the traits across temperatures (data not shown). The percentage of the total phenotypic variation attributable to genetic factors (line effects and line by temperature interaction) ranged from 41 to 91% (Fig. 2) wherein Larval Developmental Time presented, in average, more natural genetic variation, whereas Pupal Developmental Time showed the opposite pattern. Interestingly the line by temperature interaction, which stands for genotype by environment interaction, was the component of variance with a higher contribution to the total phenotypic variance for all traits analyzed. In this sense, the estimated cross-temperature genetic correlations ($rGxE_{(17-25)}$) for each combination trait-population ranked from −0.03 to 0.56 (Table 3). These data were different from unity, underscoring thermal-specific differences in all traits analyzed and suggesting that the genetic factors underpinning phenotypic variation in each trait differ between temperatures in lines collected in Raleigh, Lavalle and Uspallata.

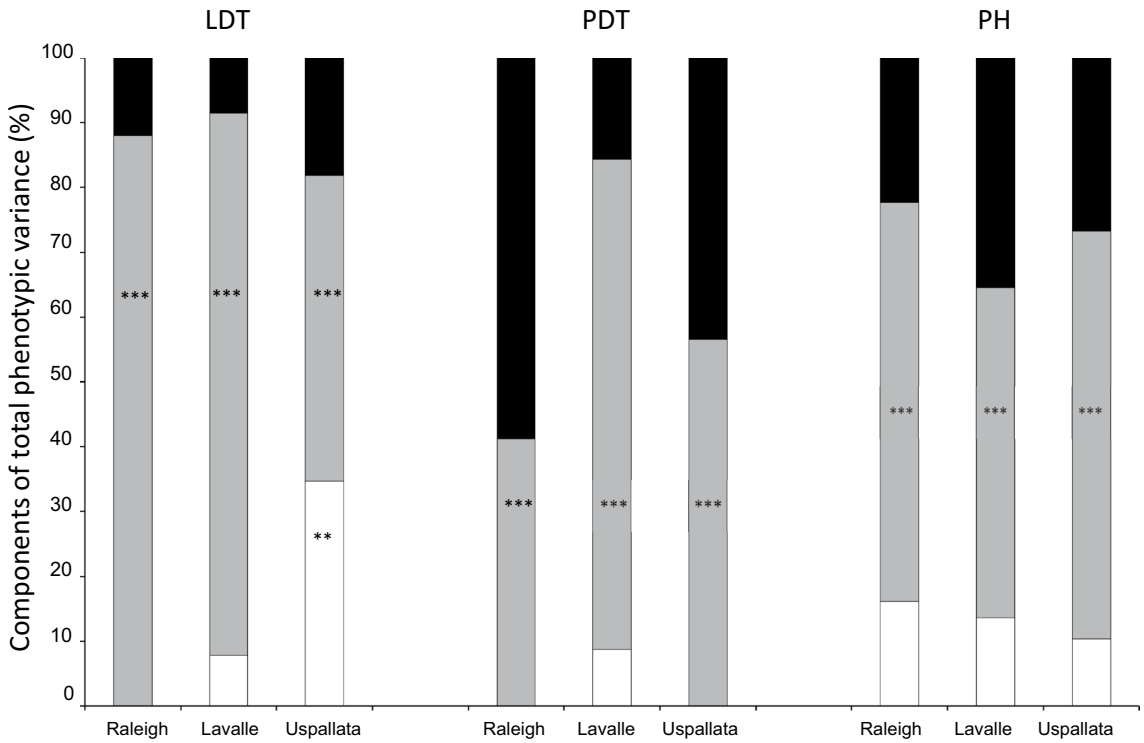


Fig. 2 Components of total phenotypic variance (in percentage): natural genetic variation (line effect, white block), genotype by environment interaction (line by temperature interaction, grey block) and the error (black block) for Larva Developmental Time (LDT), Pupa Developmental Time (PDT) and Pupation Height (PH). The analyses were performed in lines derived from Raleigh, Lavalle and Uspallata that were reared at 17 °C and 25 °C. Significant factors are represented as $^{**}p < 0.01$, and $^{***}p < 0.001$

Table 3 Estimates of quantitative genetic parameters of larval developmental time (LDT), pupal developmental time (PDT) and pupation height (PH) in flies collected from Raleigh, Lavalle and Uspallata

	LDT			PDT			PH		
	Raleigh	Lavalle	Uspallata	Raleigh	Lavalle	Uspallata	Raleigh	Lavalle	Uspallata
σ^2_G	517.12	1794.53	1219.89	73.87	929.54	232.89	25.97	17.09	28.52
σ^2_E	135.79	169.25	269.83	105.31	172.89	178.84	7.46	9.40	10.37
σ^2_P	652.92	1963.78	1489.73	179.18	1102.43	411.73	33.43	26.49	38.89
H^2	0.79	0.91	0.82	0.41	0.84	0.57	0.78	0.65	0.73
H^2_{17}	0.75	0.92	0.83	0.39	0.86	0.54	0.73	0.63	0.66
H^2_{25}	0.90	0.87	0.78	0.62	0.73	0.62	0.82	0.67	0.80
CV_G	9.45	18.89	16.53	5.60	20.51	9.65	48.92	39.70	61.79
CV_E	4.84	5.80	7.77	6.69	8.85	8.45	26.23	29.45	37.17
$rGxE_{(17-25)}$	−0.03	0.10	0.56	-0.01	0.16	>0.01	0.20	0.12	0.14

σ^2_G, σ^2_E and σ^2_P indicate the genetic, environmental and phenotypic variances respectively; CV_G, CV_E and H^2 indicate coefficients of genetic variance, coefficients of environmental variance and broad sense heritability, respectively. $rGxE_{(17-25)}$ stand for the cross temperature genetic correlations

To gain insights into the differences in aspects of the genetic architecture of Larval and Pupal Developmental Time and Pupation Height in the different populations, we calculated quantitative genetic parameters (Table 3). Estimates for genetic variance (σ^2_G) were high for all populations and traits. It is of interest to note that the values for genetic variance were lower for Pupation Height. The high heritability (H^2) observed for all traits studied can be attributable to the relatively low environmental variance (σ^2_E) for all population and traits when σ^2_E is compared with σ^2_G (Table 3). Estimates of evolvability (CV_G) for the three populations ranged from 9.45 to 18.91, from 5.60 to 20.52 and from 39.70 to 61.79 for Larval Developmental Time, Pupal Developmental Time and Pupation Height, respectively

(Table 3). These data indicate that there is potential for evolutionary adaptation to the thermal environment variation for all trait-population combinations.

The correlation analyses between traits performed for each temperature and population showed mostly genetic independence both between characters for the temperatures evaluated and between temperatures for each character studied (Table 4). Certainly, the 3 significant correlations detected (after Bonferroni correction) were population-specific and did not involve the same pairs of traits. Particularly interesting was the result obtained for the correlation analyses between Larval and Pupal Developmental Time, where only lines derived from Lavalle exhibited a significant (negative) correlation when flies developed at 17 °C, after Bonferroni correction (Fig. 3; Table 4). The non-significant genetic correlation between Larval and Pupal Developmental Time for Raleigh and Uspallata populations at the two temperatures assayed and for flies from Lavalle reared at 25 °C (Fig. 3) reveals that the genetic architecture of Developmental Time in these ontogenetic stages could be different. In order to provide evidence about a genetic decoupling between Larval and Pupal Developmental Time we analyzed the relationship between both ontogenetic stages through the Larval DT/Pupal DT ratio (see Materials and methods). Thus, if these two traits were genetically correlated, Larval DT/Pupal DT ratio should not be different, neither across populations nor among lines within populations. Our results indicate that Larval DT/Pupal DT ratio varied significantly between populations ($F_{2,210} = 7.8$, $p < 0.001$), wherein Uspallata exhibited a lower ratio compared with the other populations (Tukey test $p < 0.01$, data not shown), while thermal plasticity (temperature effect) and genetic variation for thermal plasticity (population by temperature interaction) showed no significant differences. Particularly, the absence of significant thermal plasticity and genetic variation for thermal plasticity indicates that the ratio of Developmental Time between ontogenetic stages was independent on the temperature at which the individuals were reared, and that this pattern was the same for all populations studied. Likewise, if these two traits (Larval and Pupal Developmental Time) were genetically decoupled, we would expect high differences among lines. Certainly, the analyses performed in each population reveal significant line and/or line by temperature interaction effects for this ratio for all of them (Fig. 4), indicating that the relation between Larval and Pupal Developmental Time varies among lines. Moreover, the significant line by temperature interaction can be attributable to the low cross-temperature genetic correlations ($rGxE_{(17-25)}$), suggesting that the patterns of variation for the relation between Larval and Pupal Developmental Time observed among lines for each population depend on the temperature of development (Fig. 5). Thus, the result obtained in the correlation analysis, in agreement with the significant variation exhibited for the Larval DT/Pupal DT ratio not only among but within populations and also between temperatures for each population, reveal that the genetic architectures for Developmental Time in these two ontogenetic stages are decoupled.

Table 4 Principal results of the genetic correlation analyses for larva developmental time (LDT), pupa developmental time (PDT) and pupa height (PH) among isogenic lines derived from Raleigh (R), Lavalle (L) and Uspallata (U)

	LDT	PDT	PH
LDT	**R −0.03**	R −0.22	R −0.53**
	L 0.17	L −0.61**	L −0.05
	U 0.59**	U −0.14	U −0.25
PDT	R 0.17	**R −0.02**	R −0.61
	L −0.43*†	**L 0.12**	L 0.11
	U −0.17	**U −0.01**	U 0.25
PH	R −0.30	R 0.04	**R 0.19**
	L −0.22	L −0.36*†	**L 0.19**
	U −0.12	U 0.23	**U 0.14**

r-Values are shown for each trait between temperatures (in bold on the diagonal), between each pair of traits within 17 °C (above de diagonal) and between each pair of traits within 25 °C (below de diagonal)

Significant r-values are indicated as *$p < 0.05$, **$p < 0.001$

†Not significant after Bonferroni correction for multiple tests ($P_B = 0.01$)

Discussion

Studying the evolution of genetic architecture of developmental adaptive traits is essential to understand how populations adapt to different environments (Mackay 2001; Hansen 2006). Here we compared different aspects related to genetic architecture between three natural populations of *D. melanogaster* in individuals reared at different temperatures. These populations are geographically separated (Argentinians populations vs. Raleigh population) and also differ in altitude (a highland population [Uspallata] vs. two lowland populations [Raleigh and Lavalle]). Our results revealed significant phenotypic variation among populations in the three adaptive developmental traits Larval and Pupal Developmental Time and Pupation Height and highly significant variation within populations for all traits, suggesting that they exhibit local adaptation and each population still contains significant genetic variation affecting all of them. However, the detected variation does not follow a pattern consistent with a single causal variable, suggesting that geographical (limits to the genetic flow) and/or ecological (adaptation to environmental variables) factors would be responsible for the differences between populations. Given the relatively

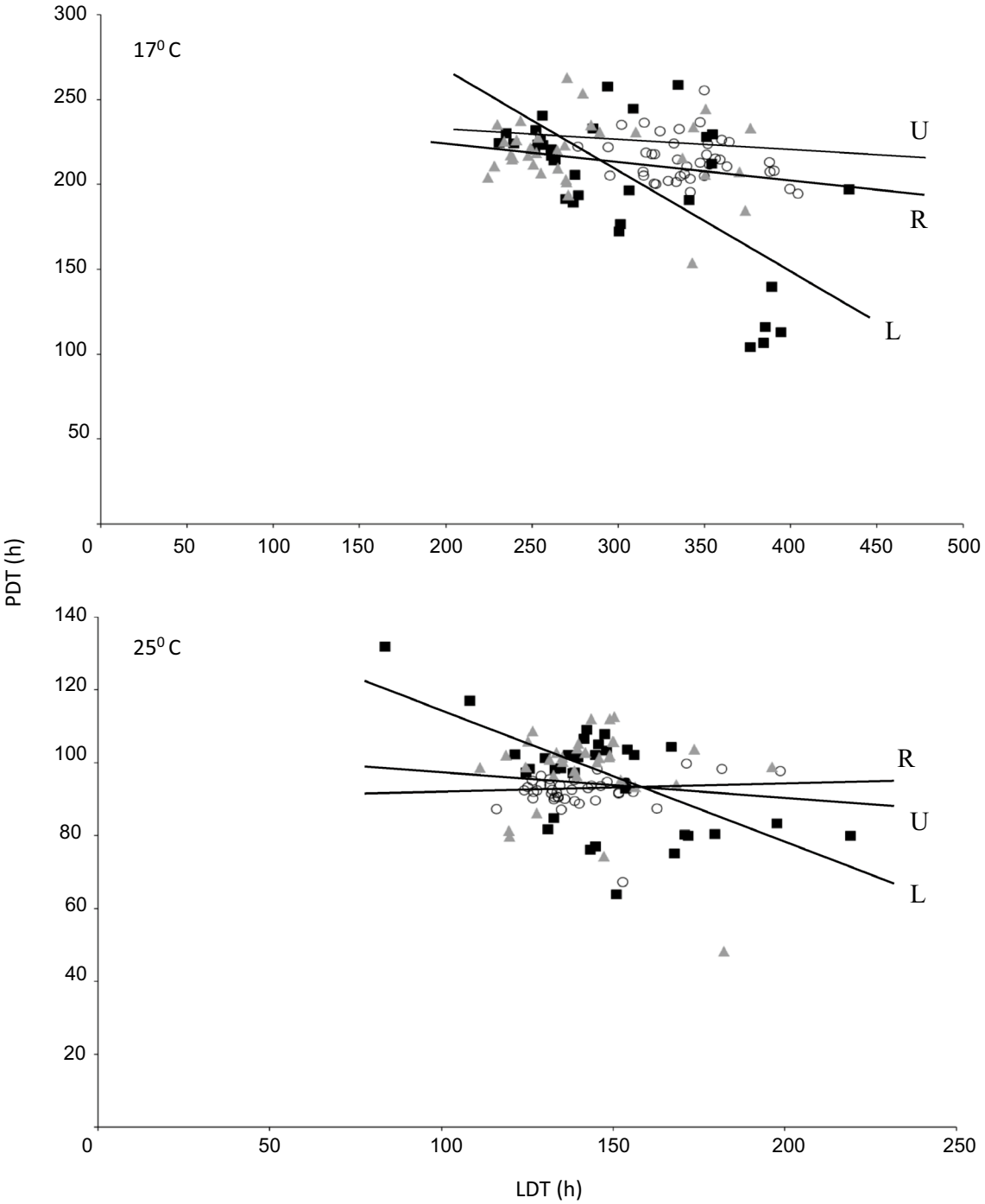


Fig. 3 Correlations between Larval Developmental Time (LTD) and Pupal Developmental Time (PDT) in hours for lines derived from Raleigh (R, open circle), Lavalle (L, filled square) and Uspallata (U, grey triangle) reared at 17 °C and 25 °C. Trend line for each correlation between LTD and PDT are showed when lines derived from Raleigh (R), Lavalle (L) and Uspallata (U) were reared at 17 °C and 25 °C

constant population sizes (see Materials and Methods), significant effects of genetic drift would be unlikely.

Theoretical arguments and empirical data (Mousseau and Roff 1987; Falconer and Mackay 1996; Houle 1992; Lavagnino et al. 2008) have addressed that natural selection rapidly exhausts additive genetic variance for fitness traits compared with morphological traits, and, therefore, that populations are likely to harbor little additive genetic variance for fitness related traits. Our estimates of quantitative genetic parameters revealed high genetic variance (σ^2_G) and

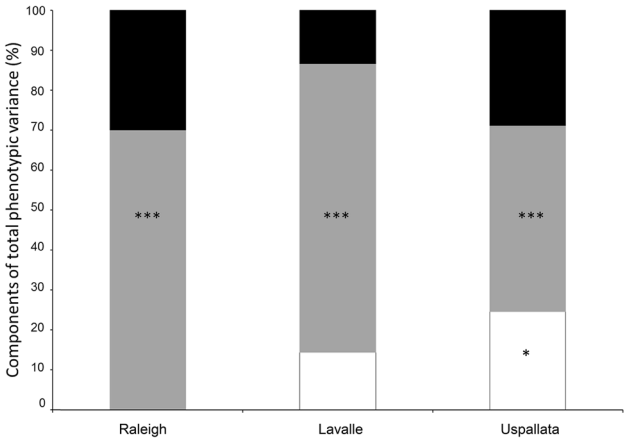


Fig. 4 Components of total phenotypic variance (in percentage): natural genetic variation (line effect, white block), genotype by environment interaction (line by temperature interaction, grey block) and the error (black block) for the log LDT/PDT ratio. The analysis were performed in lines derived from Raleigh, Lavalle and Uspallata that were reared at 17 °C and 25 °C. Significant factors are represented as *$p < 0.05$, and ***$p < 0.001$. †Not significant after Bonferroni correction for multiple tests ($P_B = 0.01$)

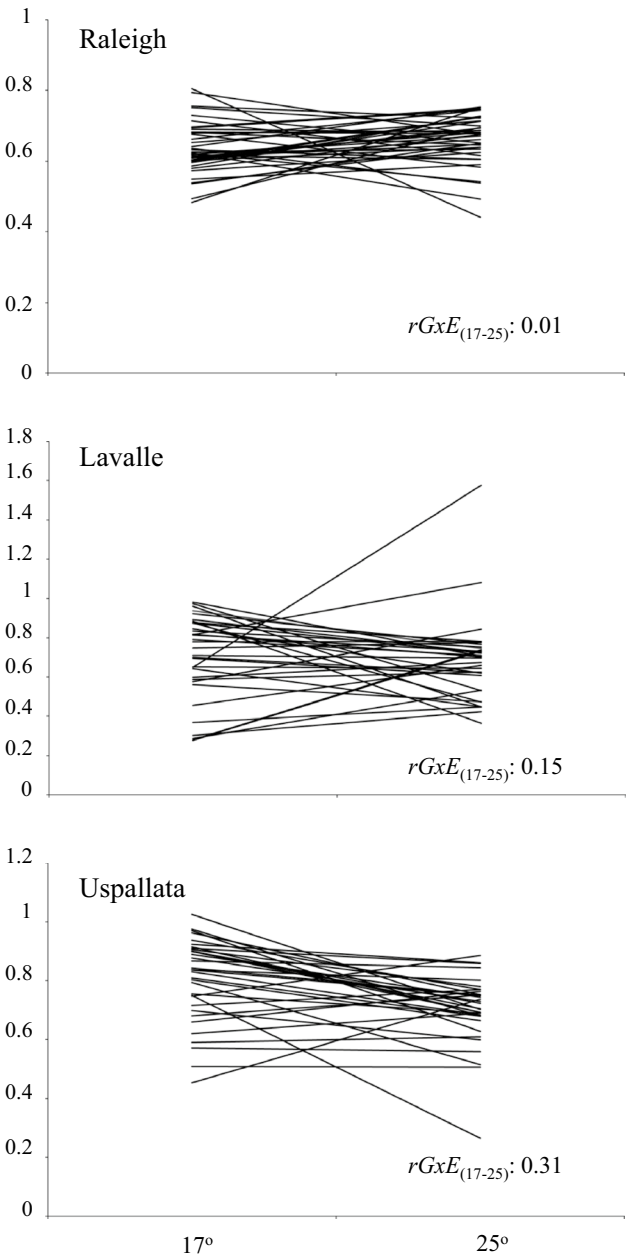


Fig. 5 Norms of reaction for the log LDT/PDT ratio in lines derived from Raleigh, Lavalle and Uspallata populations reared at 17 °C and 25 °C. The genetic correlations between temperatures ($rGxE_{(17–25)}$) for each population is indicated

heritability (H^2) for all traits and populations, suggesting substantial genetic variation affecting both components of Developmental Time and Pupation Height. We have not detected a clear trend regarding the differences between populations for the quantitative genetic parameters, given that no population presented the highest or lowest values for these components. In a previous study, Mensch et al. (2010) observed that second chromosome substitution lines derived from Lavalle and Uspallata populations exhibited also considerable genetic variation for total Developmental Time (from first instar larva to adult). In this sense, it is important to note that the lines collected in Lavalle and Uspallata in the analysis performed by Mensch et al. (2010) and in our study were different. Thus, two independent studies showed that Developmental Time exhibits high heritability, suggesting that this adaptive trait harbors sizable natural genetic variation.

As expected, thermal plasticity was found for all trait and population combinations. Interestingly, the populations showed less differences in Developmental Time traits at 25 °C, which may be explained either by the existence of trade-offs with another traits [as in the aforementioned fast development syndrome (Chippindale et al. 2004)] or, by indirect selection or epigenetic changes during the isogenization procedures, carried out at 25 °C. These temperature-specific differences in the genetic architecture of the traits imply the potential for their independent evolution at each temperature. However, given that natural populations should cope with daily and seasonal temperature changes probably associated to constantly shifting phenotypic optima, the loss of genetic variation affecting these traits would be reduced (Sasaki and Ellner 1997; Bürger and Gimelfarb 2002), explaining the importance of the genetic components underlying phenotypic variability and the high heritability of the traits.

In this study, we found significant differences in the correlation analyses between Laval and Pupal Developmental Time in 2 out of 3 populations, and besides, significant differences between and within populations for the Larval DT/

Pupal DT ratio, suggesting that Larval and Pupal Developmental Times are decoupled. Even though these stages are characterized by different behaviors, morphologies and distinct processes and genetic programs underlying them, the possible decoupling between Larval and Pupal Developmental Time has been frequently overlooked, although it has been addressed separately in a few other studies (Partridge and Fowler 1992; Partridge et al. 1994; Chippindale et al. 2003; Artieri and Singh 2010).

The fact that we detected decoupling in developmental timing suggests that larval and pupal traits may have different underlying genetic architectures. In this sense, Folguera et al. (2010) demonstrated that larvae and pupae may choose alternative "decisions" when confronted to thermal variation. These "decisions" are general "rules" that have stage-specific genetic bases which specify how an actor, in this case an individual in a given ontogenetic stage, should respond to environmental change (Folguera et al. 2010). In fact, diverse studies revealed that the transcriptional patterns differ throughout ontogeny (Arbeitman et al. 2002; Artieri and Singh 2010; Gerstein et al. 2014), suggesting stage-specific gene regulatory networks and consequently different genetic architectures. Furthermore, Mensch et al. (2010) demonstrated that four candidate genes (*invected, mastermind, cricklet* and *CG14591*), associated with natural phenotypic variation for Developmental Time, have allelic variants that affected Larval but not Pupal Developmental Time.

Modularity may have enabled holometabolous insects to optimize life-history components, such as growth and reproduction, through temporal partitioning (Bryant 1969; Ebenman 1987; Moran 1994; Truman and Riddiford 1999; Minelli and Fusco 2010; McMahon and Hayward 2016). Certainly, our results provide evidence that developmental timing for different stages could have evolved a genetic decoupling, so that independent changes occur in Larval and Pupal Developmental Time. The uncoupling between the different ontogenetic levels of a trait could influence different aspects of the phenotype, including means, plasticity, canalization, plasticity of canalization, contributing to the adoption of different adaptive strategies and favoring the maintenance of genetic variability (Lavagnino and Fanara 2016; Melo et al. 2016). Indeed, the high genetic variation that we detected affecting all traits can partially be attributable to these decouplings. In conclusion, we underscore the need to consider ontogeny as a very complex set of interrelated processes but contemplating at the same time the modularity between these stages characterized by different behaviors and relationships with the environment (Wagner et al. 1997). We propose that Larval and Pupal Developmental Times should be addressed separately, avoiding the loss of large amount of useful information that allows a better understanding of the different processes occurring during development and their complex variations (Nunney 1996, 2007; Kingsolver and Huey 2008). Certainly, the decoupling allows comprehending the roles of the genotype and the environment affecting these ontogenetic stages and how their variability can modify the adult phenotype. Future studies will be carried out to analyze if the genetic variants underpinning natural genetic variation for these traits differ between populations and also according to the stage and temperature conditions assessed.

Acknowledgements This work was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (FONCyT, PICT) and Consejo Nacional de Ciencia y Técnica (CONICET). MAPZ and VEO are recipients of doctoral scholarships from CONICET (Argentina) and JJF is a member of Carrera del Investigador Cientifico of CONICET (Argentina).

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

References

Angilletta, M., Steury, T., & Sears, M. (2004). Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. *Integrative and Comparative Biology, 44*, 498–509.

Arbeitman, M. N., Furlong, E. E., Imam, F., Johnson, E., Null, B. H., et al. (2002). Gene expression during the life cycle of *Drosophila melanogaster. Science, 297*, 2270–2275.

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*, 26.

Atchley, W. R., Gaskins, C. T., & Anderson, D. (1976). Statistical properties of ratios. I. Empirical results. *Systematic Biology, 25*, 137–148.

Bauer, S. J., & Sokolowski, M. B. (1985). A genetic analysis of path length and pupation height in a natural population of *Drosophila melanogaster. Canadian Journal of Genetics and Cytology, 27*, 334–340.

Beltramí, M., Medina-Muñoz, M. C., Arce, D., & Godoy-Herrera, R. (2010). *Drosophila* pupation behavior in the wild. *Evolutionary Ecology*, *24*, 347–358.

Bharathi, N. S., Prasad, N. G., Shakarad, M., & Joshi, A. (2004). Correlates of sexual dimorphism for dry weight and development time in five species of *Drosophila. Journal of Zoology, 264*, 87–95.

Bryant, E. H. (1969). A system favoring evolution of holometabolous development. *Annals of the Entomological Society of America, 62*, 1087–1091.

Bürger, R., & Gimelfarb, A. (2002). Fluctuating environments and the role of mutation in maintaining quantitative genetic variation. *Genetics Research, 80*, 31–46.

Carreira, V. P., Imberti, M., Mensch, J., & Fanara, J. J. (2013). Gene-by-temperature interactions and candidate plasticity genes for morphological traits in *Drosophila melanogaster. PLoS ONE, 8*(7), e70851.

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

Chippindale, A. K., Alipaz, J. A., Chen, H. W., & Rose, M. R. (1997). Experimental evolution of accelerated development in Drosophila.

1. Developmental speed and larval survival. *Evolution, 51*(5), 1536–1551.

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 & M. Matos (eds.), *Methuselah flies: A case study in the evolution of aging* (pp. 413–435). Singapore: World Scientific Press.

Chippindale, A. K., Ngo, A. L., & Rose, M. R. (2003). The devil in the details of life-history evolution: Instability and reversal of genetic correlations during selection on *Drosophila* development. *Journal of Genetics, 82*, 133–145.

Conner, J. C., & Hartl, D. L. (2004). *A primer of ecological genetics*. Sunderland: Sinauer Associates Incorporated.

Davidowitz, G., & Nijhout, H. F. (2004). The physiological basis of reaction norms: The interaction between growth rate, the duration of growth and body size. *Integrative and Comparative Biology, 44*, 443–449.

Del Pino, F., Jara, C., Pino, L., & Godoy-Herrera, R. (2014). The neuro-ecology of *Drosophila* pupation behavior. *PLoS ONE*, *9*(7), e102159.

Del Pino, F., Salgado, E., & Godoy-Herrera, R. (2012). Plasticity and genotype x environment interactions for locomotion of *Drosophila melanogaster* larvae. *Behavior Genetics, 42*, 162–169.

DeWitt, J., & Scheiner, S. M. (2004). *Phenotypic plasticity: Functional and conceptual approaches*. Oxford: Oxford University Press.

Dillon, M., Wang, G., Garrity, P. A., & Huey, R. B. (2009). Thermal preference in *Drosophila. Journal of Thermal Biology, 34*, 109–119.

Ebenman, B. (1987). Niche differences between age classes and intraspecific competition in age-structured populations. *Journal of Theoretical Biology, 124*, 25–33.

Edgar, B. A. (2006). How flies get their size: Genetics meets physiology. *Nature Reviews Genetics, 7*, 907–916.

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics* (4th ed.). Essex: Longman.

Fallis, L. C., Fanara, J. J., & Morgan, T. J. (2014). Developmental thermal plasticity among *Drosophila melanogaster* populations. *Journal of Evolutionary Biology, 27*, 557–564.

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.

Fanara, J. J., & Hasson, E. (2001). Oviposition acceptance and fecundity schedule in the cactophilic sibling species *Drosophila buzzatii. and D. koepferae on their natural hosts. Evolution, 55*, 2615–2619.

Flatt, T. (2005). The evolutionary genetics of canalization. *The Quarterly Review of Biology, 80*, 287–316.

Flatt, T., & Heyland, A. (2011). *Mechanisms of life history evolution: The genetics and physiology of life history traits and trade-offs*. Oxford: Oxford University Press.

Folguera, G., Mensch, J., Muñoz, J. L., Ceballos, S. G., Hasson, E., & Bozinovic, F. (2010). Ontogenetic stage-dependent effect of temperature on developmental and metabolic rates in a holometabolous insect. *Journal of Insect Physiology, 56*, 1679–1684.

Gerstein, M. B., Rozowsky, J., Yan, K. K., Wang, D., Cheng, C., et al. (2014). Comparative analysis of the transcriptome across distant species. *Nature, 512*, 445–448.

Gilchrist, G. W., Jeffers, L. M., West, B., Folk, D. G., Suess, J., & Huey, R. B. (2008). Clinal patterns of desiccation and starvation resistance in ancestral and invading populations of *Drosophila subobscura. Evolutionary Applications, 1*, 513–523.

Goldstein, D. B., & Pollock, D. D. (1997). Launching microsatellites: A review of mutation processes and methods of phylogenetic inference. *Journal of Heredity, 88*, 335–342.

Hansen, T. F. (2006). The evolution of genetic architecture. *Annual Review of Ecology, Evolution, and Systematics, 37*, 123–157.

Houle, D. (1992). Comparing evolvability and variability of quantitative traits. *Genetics, 130*, 195–204.

Huang, W., Massouras, A., Inoue, Y., Peiffer, J., Ràmia, M., et al. (2014). Natural variation in genome architecture among 205 *Drosophila melanogaster* genetic reference panel lines. *Genome Research, 24*, 1193–1208.

Jumbo-Lucioni, P., Ayroles, J. F., Chambers, M. M., Jordan, K. W., Leips, J., Mackay, T. F., & De Luca, M. (2010). Systems genetics analysis of body weight and energy metabolism traits in *Drosophila melanogaster. BMC Genomics, 11*, 297.

Kingsolver, J. G., & Huey, R. B. (2008). Size, temperature, and fitness: Three rules. *Evolutionary Ecology Research, 10*, 251–268.

Lavagnino, N., Anholt, R. R. H., & Fanara, J. J. (2008). Variation in genetic architecture of olfactory behavior among wild-derived populations of *Drosophila melanogaster. Journal of Evolutionary Biology, 21*, 988–996.

Lavagnino, N., & Fanara, J. J. (2016). Changes across development influence visible and cryptic natural variation of *Drosophila melanogaster* olfactory response. *Evolutionary Biology, 43*, 96–108.

Long, T. A. F., Pischedda, A., Stewart, A. D., & Rice, W. R. (2009). A cost of sexual attractiveness to high-fitness females. *PLoS Biology, 7*(12), e1000254.

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits* (p. 663). Sunderland: Sinauer Associates, Inc.

Mackay, T. F. C. (2001). The genetic architecture of quantitative traits. *Annual Review of Genetics, 35*, 303–339.

Mackay, T. F. C., Richards, S., Stone, E. A., Barbadilla, A., Ayroles, J. F., et al. (2012). The *Drosophila melanogaster* genetic reference panel. *Nature, 482*, 173–178.

Markow, T. A. (1979). A survey of intra- and interspecific variation for pupation height in *Drosophila. Behavior Genetics, 9*, 209–217.

Matzkin, L. M., Watts, T. D., & Markow, T. A. (2007). Desiccation resistance in four *Drosophila* species: Sex and population effects. *Fly, 1*, 268–273.

McMahon, D. P., & Hayward, A. (2016). Why grow up? A perspective on insect strategies to avoid metamorphosis. *Ecological Entomology, 41*, 505–515.

Melo, D., Porto, A., Cheverud, J. M., & Marroig, G. (2016). Modularity: Genes, development, and evolution. *Annual Review of Ecology, Evolution, and Systematics, 47*, 463–486.

Mensch, J., Carreira, V. P., Lavagnino, N., Goenaga, J., Folguera, G., Hasson, E., & Fanara, J. J. (2010). State-specifics effects of candidate heterochronic genes on variation in developmental time along an altitudinal cline of *Drosophila melanogaster. PLoS ONE, 5*(6), e11229.

Mensch, J., Lavagnino, N., Carreira, V. P., Massaldi, A., Hasson, E., & Fanara, J. J. (2008). Identifying candidate genes affecting developmental time in *Drosophila melanogaster*: Pervasive pleiotropy and gene-by-environment interaction. *BMC Developmental Biology, 8*, 78.

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*, 373–383.

Minelli, A., & Fusco, G. (2010). Developmental plasticity and the evolution of animal complex life cycles. *Philosophical Transactions of the Royal Society B, 365*, 631–640.

Mirth, C. K., & Riddiford, L. M. (2007). Size assessment and growth control: How adult size is determined in insects. *Bioessays, 29*, 344–355.

Moran, N. (1994). Adaptation and constraint in the complex life cycles of animals. *Annual Review of Ecology and Systematics, 25*, 573–600.

Mousseau, T. A., & Roff, D. A. (1987). Natural selection and the heritability of fitness components. *Heredity, 59*, 181–197.

Mueller, L. D., & Sweet, V. F. (1986). Density-dependent natural selection in *Drosophila*: Evolution of pupation height. *Evolution, 40*, 1354–1356.

Narasimha, S., Kolly, S., Sokolowski, M. B., Kawecki, T. J., & Vijendravarma, R. K. (2015). Prepupal building behavior in *Drosophila melanogaster* and its evolution under resource and time constraints. *PLoS ONE, 10*, e0117280.

Nunney, L. (1996). The response to selection for fast larval development in *Drosophila melanogaster* and its effect on adult weight: An example of a fitness trade-off. *Evolution, 50*, 1193–1204.

Nunney, L. (2007). Pupal period and adult size in *Drosophila melanogaster*: A cautionary tale of contrasting correlations between two sexually dimorphic traits. *Journal of Evolutionary Biology, 20*, 141–151.

Nylin, S., & Gotthard, K. (1998). Plasticity in life-history traits. *Annual Review of Entomology, 43*, 63–83.

Paranjpe, D. A., Anitha, D., Chandrashekaran, M. K., Joshi, A., & Sharma, V. K. (2005). Possible role of eclosion rhythm in mediating the effects of light-dark environments on pre-adult development in *Drosophila melanogaster. BMC Developmental Biology, 5*, 5.

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*, 645–663.

Partridge, L., & Fowler, K. (1992). Direct and correlated response to selection on age at reproduction in *Drosophila melanogaster. Evolution, 46*, 76–91.

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*, 1363–1372.

Rewitz, K. F., Yamanaka, N., & O'Connor, M. B. (2013). Chapter one—Developmental checkpoints and feedback circuits time insect maturation. *Current Topics in Developmental Biology, 103*, 1–33.

Riedl, C. A., Riedl, M., Mackay, T. F., & Sokolowski, M. B. (2007). Genetic and behavioral analysis of natural variation in *Drosophila melanogaster* pupation position. *Fly, 1*, 23–32.

Rodrigues, M. A., Martins, N. E., Balancé, L. F., Broom, L. N., Dias, A. J., Fernandes, A. S. D., Rodrigues, F., Sucena, E., & Mirth, C. K. (2015). *Drosophila melanogaster* larvae make nutritional choices that minimize developmental time. *Journal of Insect Physiology, 81*, 69–80.

Roff, D. A. (1992). *The evolution of life histories: Theory and analysis*. New York: Chapman & Hall.

Sasaki, A., & Ellner, S. (1997). Quantitative genetic variance maintained by fluctuating selection with overlapping generations: Variance components and covariances. *Evolution, 51*, 682–696.

Schlichting, C., & Pigliucci, M. (1998). *Phenotypic evolution: A reaction norm perspective*. Sunderland: Sinauer Associates Incorporated.

Shingleton, A. W., Das, J., Vinicius, L., & Stern, D. L. (2005). The temporal requirements for insulin signaling during development in *Drosophila. PLoS Biology, 3*(9), e289.

Singh, B. N., & Pandey, M. B. (1993). Evidence for additive polygenic control of pupation heigh in *Drosophila ananassae. Hereditas, 119*, 111–116.

Sokolowski, M. B., & Hansell, R. I. (1983). Elucidating the behavioral phenotype of *Drosophila melanogaster* larvae: Correlations between larval foraging strategies and pupation height. *Behavior Genetics, 13*, 267–280.

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional Ecology, 3*, 259–268.

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

Trotta, V., Calboli, F. C., Ziosi, M., Guerra, D., Pezzoli, M. C., David, J. R., & Cavicchi, S. (2006). Thermal plasticity in *Drosophila melanogaster*: A comparison of geographic populations. *BMC Evolutionary Biology, 6*, 67.

Truman, J. W., & Riddiford, L. M. (1999). The origins of insect metamorphosis. *Nature, 401*, 447–452.

van Noordwijk, A. J., & de Jong, G. (1986). Acquisition and allocation of resources: Their influence on variation in life history tactics. *American Naturalist, 128*, 137–142.

Wagner, G. P., Booth, G., & Bagheri-Chaichian, H. (1997). A population genetic theory of canalization. *Evolution, 51*(2), 329–347.

Welbergen, P., & Sokolowski, M. (1994). Development time and pupation behavior in the *Drosophila melanogaster* subgroup (Diptera: Drosophilidae). *Journal of Insect Behavior, 7*, 263–277.

Werenkraut, V., Hasson, E., Oklander, L., & Fanara, J. J. (2008). A comparative study of competitive ability between two cactophilic species in their natural hosts. *Austral Ecology, 33*, 663–671.

Yang, A. S. (2001). Modularity, evolvability, and adaptive radiations: A comparison of the hemi- and holometabolous insects. *Evolution & Development, 3*, 59–72.