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ORIGINAL RESEARCH

Plasticity and Genotype × Environment Interactions for Locomotion in *Drosophila melanogaster* Larvae

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Abstract Locomotion is a primary means by which animals interact with the world. To understand the contribution of genotype × environment interactions to individual differences in *D. melanogaster* larval locomotion we investigated phenotypic sensitivity to environmental changes in four strains of this species and their F_1 hybrids. We also investigated to what extent flexibility and plasticity of locomotion depend upon larval age. Specifically, we examined larval locomotion at 48 and 96 h of development on three different substrates. Locomotion was influenced by the structure of the substrate, but this depended on both the genotype and larval age. At 48 h of larval development phenotypic variation in locomotion was attributable to both genotype × environment interactions and genotypic differences among the larvae, while at 96 h of age, differences were mainly due to genotype × environment interactions. An analysis of variance of the 4 × 4 diallel cross made at 48 and 96 h of development showed, depending on the cross, either dominance to increase/decrease locomotion, overdominance to increase/decrease locomotion, or no dominance to increase/decrease locomotion. Furthermore, the diversity of behavioral responses in the F_1 hybrids was greater at 96 than at 48 h of larval development. Our results demonstrate that genotype × environment interactions result in plasticity of *D. melanogaster* larval locomotion, which makes sense in light of the fact that larvae, in the wild, develop in heterogeneous and rapidly changing environments.

Keywords *D. melanogaster* larval locomotion · Larval development · Diallel cross · Plasticity · Genotype × environment interactions

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This paper is dedicated to Dr Barrie Burnet and Dr Lynn Burnet.

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Introduction

Together with sensory systems, locomotion is the primary means by which animals interact with the world. In species as diverse as insects, mice, and humans, motor responses have evolved towards gaining access to environmental resources that increase individual fitness. Whether the animal is single-celled or multicellular activities such as foraging, escaping a stressful environment, and finding a mate all rely on motor responses. Moreover, animals living in heterogeneous environments that vary over time and space depend on locomotion to adapt to new environmental conditions (Green et al. 1983; Sokolowski et al. 1984; Beltramí et al. 2010).

Not all individuals respond to environmental changes in the same way (Burcher and Plomin 2008). Because locomotion is influenced by genes and environment, study of variation among genotypes in phenotypic sensitivity to environmental changes is a primary goal in the study of locomotion (Anholt and Mackay 2004; Sambandam et al. 2009). While some genes involved in locomotion in *D. melanogaster* larvae have been identified (de Bono and Sokoloski 2007), the role of behavioral phenotypic

plasticity in the adaptation of larvae to their natural breeding sites is not well understood. In the wild, larvae of several species of *Drosophila* develop in heterogeneous and rapidly changing environments due in part to the effects of ongoing fermentation by microorganisms in the structure of the breeding site. The decaying process is heterogeneous, as some parts of a fruit unit may be in an advanced state of decay, while fermentation is just beginning in other areas of the same fruit. Thus, larvae of *Drosophila* move searching for colonies of bacteria and yeasts, their principal source of proteins (Fogleman et al. 1981; Arizmendi et al. 2008). While *Drosophila* larvae ingest the yeasts and bacteria, they decrease locomotion (Ruiz-Dubreuil et al. 1996). However, once food sources are depleted, the larvae explore the fruit looking for new food sources. In addition, locomotion is also an essential component of searching for appropriate pupation sites. In the wild, pupae of *D. simulans* and *D. buzzatti* are found on fruit pulp, under and over fruit skin and on the land under the fruit (Beltramí et al. 2010), indicating that the larvae explore a variety of microhabitats before they select places to pupate. These ecological characteristics in variable *Drosophila* breeding sites suggest that environment and genotype × environment interactions could play an important role in the production of individual differences in locomotion.

Life histories are often complex and may involve developmental stages that are best understood when they are considered as adaptations in their own right and not merely as steps towards adulthood. For example, the development of *Drosophila* larvae includes three instars. In the first two, larval locomotion is used primarily in foraging while in the third instar locomotion is involved in the selection of pupation sites (Ashburner et al. 2005). Interestingly, individuals of the same strain but of different ages may show variation in response to the same environment (Ruiz-Dubreuil et al. 1996). Consequently, a full understanding of locomotion as an adaptive trait must consider the effects of age and environment on this behavior (Gould 1977).

One pair of alleles of the *foraging* locus controls ingestion of food in larvae and adults of *D. melanogaster* (de Bono and Sokolowski 2007). Rover larvae draw long foraging trails, while sitter larvae follow short ones. The *foraging* (*for*) locus encodes a cGMP-dependent protein kinase (PKG); rover larvae have higher PKG enzyme activity than sitter larvae (Osborne et al. 1997). In *D. melanogaster* Ruiz-Dubreuil and del Solar (1983) genetically selected for high (HA) and low (LA) egg aggregation for more than 360 generations. They found that locomotion of larvae of the LA line was lower than in the HA line, and that LA larvae exhibit a more flexible pattern of foraging than HA larvae. In *Drosophila funebris*, larval locomotion differs between natural populations and epistasis and dominance are involved in the control of this behavior (Arizmendi et al. 2008). In adults of *D. melanogaster*, about 50% of phenotypic variation in locomotion is attributable to genotype × environment interactions (Sambandam et al. 2009).

In these studies we ask whether locomotion of *Drosophila* larvae is a flexible behavior that responds to environmental changes, and whether this plasticity is related to larval-age and/or genetic background. Specifically, our proposition is that the degree of plasticity of *D. melanogaster* larval locomotion changes with age, and that the genotypes differ in their sensitivity to environmental change. Moreover, the magnitude of genotype × environment interactions in larval locomotion of *D. melanogaster* may influence larval performance within their natural breeding sites, and thus it may be a reliable indicator of adaptation to spatially and temporally variable environments. To test this hypothesis, we recorded locomotion of larvae of 48 and 96 h of age in three different environmental conditions. We also performed a genetic analysis of locomotion at 48 and 96 h of larval age in three environments, by using a 4 × 4 diallel cross. We were specifically interested in whether genetic architecture of the trait changed in relation to the type of substrate on which larvae moved estimating the magnitudes of additive and dominance components, and epistasis in the different environments.

Materials and methods

Subjects

The stocks of *D. melanogaster* used were the Oregon R–c (wild type strain), *scarlet*, *brown* and *yellow* strains. The mutations were chosen at random from our strain collection: they came from Columbia University (USA) arriving in the University of Chile in 1952 brought by Professor Danko Brncic. We were not interested in knowing whether the mutations *per se* affected the behavior studied here. In the present experiments, we presumed that the mutations were merely phenotypic markers to distinguish one stock from another. All strains were maintained by mass culture in 0.24-l bottles containing about 50 ml of Burdick's medium (1954). All stocks were kept at 24°C under constant light, since facilities to change the light/dark cycle were not available in the laboratory.

Collection of larvae

We randomly selected groups of 60–70 inseminated 3-day-old females of the Oregon R–c, *brown*, *scarlet* and *yellow*

strains. We allowed them to lay eggs for 3–4 h on plastic spoons filled with Burdick's medium spread with fresh live yeast. We measured locomotion of 48 and 96 h old larvae. These times were chosen because *D. melanogaster* larvae show a switch from feeding activity at 48 h to cessation of the behavior and the start of the wandering phase that precedes pupation. This typically occurs after 96 h (Godoy-Herrera et al. 1984; Sokolowski et al. 1984). The measurements were made at the same time of the day for all strains and F_1 hybrid larvae.

Locomotion on three substrates

In the wild, *D. melanogaster* larvae move on heterogeneous substrates while searching for food and pupation sites (Beltramí et al. 2010). Thus, we recorded locomotion on three laboratory substrates: agar (3% in plain water), *Drosophila* Burdick's medium, and agar covered with a film of yeast suspension (40% in plain water). The texture of these substrates differs, as the surface of agar is smooth and moist, *Drosophila* medium is less moist and its surface in not smooth as agar, and agar covered with yeast paste creates a substrate that resembles to fruit pulp.

For each substrate, we examined larval locomotion on Petri dishes (9.0 cm in diameter). We deposited larvae individually onto the centre of the Petri dishes, and gave them 1 min to acclimate. We then recorded larval locomotion for 2 min using a camera lucida. Length of the track made by each larva was measured using a Hoffritz Map Measurer, and distances were converted into cm. We examined locomotion of 25 individuals for each substrate. Each larva was measured once and then discarded together with its corresponding Petri dish.

Diallel crosses

For the diallel cross, virgin males and females of the Oregon R–c, *brown*, *scarlet* and *yellow* strains were crossed in all possible combinations, and locomotion of 25 larvae of 48 and 96 h of development was recorded on the three substrates, as described above. Thus, the genetic analysis was based on 25 replicate larvae per cell (400 larvae per larval age and substrate combination). The corresponding diallel tables at 48 and 96 h of larval development were analyzed following Mather and Jinks (1971).

To test whether an additive-dominance model with additive environmental effects and without epistasis fit our data (see Table 1), we performed an analysis of the relationship between the variance (V_r) and parent-offspring covariance (W_r) for members of the same array. In the absence of non-allelic interaction and with independent distribution of the genes among parents W_r is related to V_r by a straight line of unit slope (Mather and Jinks 1971).

Additional statistical analysis

To describe individual differences in locomotion of larvae of 48 and 96 h old within the Oregon R–c, *brown*, *scarlet* and *yellow* strains, we estimate magnitudes of environmental and genotype × interaction effects by applying a linear function of four components (Lynch and Walsh 1998):

$$Z_{ijk} = G_i + I_{ij} + E_j + e_{ijk}$$

Zijk locomotion of *k*th larva of *i*th genotype crawling on *j*th substrate, *Gi* expected locomotion of the particular genotype *i* averaged over the three substrates, *Iij* genotype × environment interaction effects on larval locomotion, *Ej* and *eijk* general and specific environmental effects to larval locomotion

Results

Table 1 shows locomotion of parental and F_1 hybrid larvae of 48 and 96 h of age. The three entries in each cell of the Table are locomotion on agar, *Drosophila* culture medium, and food-agar covered with a film of yeast, respectively. The larvae were obtained by reciprocal cross the Oregon R–c, *brown*, *yellow* and *scarlet* strains. On agar and *Drosophila* medium, F_1 hybrid larvae of 48 h of age showed higher locomotion than those of the parental larvae (e.g., Oregon R–c × *scarlet* reciprocal hybrid larvae, G test of independence with respect to *scarlet* parent = 5.24 (Oregon R–c × *scarlet*) and 6.05 (*scarlet* × Oregon R–c), $df = 1$; $p < 0.05$). In the presence of food, parental and hybrid larvae showed similar locomotion (Table 1).

At 96 h of age, on agar, hybrid larvae showed locomotion higher than that of the parental generation, as seen, for example, with Oregon R–c × *scarlet* hybrid larvae (G-test of independence with respect to *scarlet* larvae = 6.32 (Oregon R–c × *scarlet*) and 6.04 (*scarlet* × Oregon R–c); $df = 1$; $p < 0.5$). However, on *Drosophila* medium, locomotion of F_1 hybrid larvae was lower than that of parental larvae (e.g., Oregon R–c × *scarlet* hybrid larvae, G-test = 5.09 and 4.98; respectively; $p < 0.05$; Table 1). In fact, on *Drosophila* medium mean locomotion of F_1 hybrid larvae of 96 h of age is 3.34 ± 0.07 cm lower than mean locomotion of parent larvae. In the presence of food, hybrid and parental larvae of 96 h of age showed similar locomotion (G-test, $p > 0.05$ (Table 1). We conclude that 96 h-old larvae are more sensitive than 48 h-old larvae to physical differences of the substrates.

Table 1 Locomotion of larvae of 48 and 96 h of age in a diallel set of matings among the Oregon R–c (wild type), brown, yellow and scarlet strains

Females	Males			
	Oregon R–c	Brown	Yellow	Scarlet
(a) 48-hour-old larvae				
Oregon R–c	5.61 ± 0.78	5.60 ± 1.04	6.26 ± 0.92	5.32 ± 1.36
	4.79 ± 1.20	7.41 ± 1.52	5.69 ± 2.41	8.78 ± 1.38
	5.33 ± 2.33	5.89 ± 0.24	8.84 ± 0.65	5.30 ± 0.18
Brown	6.01 ± 0.96	4.72 ± 0.35	7.75 ± 2.07	6.61 ± 0.52
	13.75 ± 2.65	7.64 ± 2.32	8.61 ± 1.85	5.00 ± 0.02
	6.89 ± 1.01	6.12 ± 2.05	7.30 ± 0.23	4.66 ± 1.67
Yellow	5.81 ± 0.72	6.25 ± 1.22	4.47 ± 1.78	6.06 ± 2.07
	8.81 ± 2.31	5.59 ± 1.05	7.84 ± 0.56	5.52 ± 0.48
	7.27 ± 2.08	5.10 ± 0.05	7.05 ± 0.34	4.25 ± 0.56
Scarlet	5.41 ± 1.07	5.98 ± 1.25	6.91 ± 0.71	5.95 ± 2.45
	8.47 ± 1.23	4.73 ± 1.72	4.21 ± 1.69	4.83 ± 0.03
	5.07 ± 0.37	5.13 ± 0.61	7.12 ± 0.36	6.98 ± 2.56
(b) 96-hour-old larvae				
Oregon R–c	12.96 ± 2.34	15.37 ± 1.26	16.58 ± 2.45	14.34 ± 2.35
	11.95 ± 0.28	8.03 ± 1.39	6.76 ± 0.34	13.29 ± 3.67
	15.14 ± 1.28	7.64 ± 2.67	13.81 ± 3.71	17.77 ± 2.89
Brown	13.17 ± 2.35	15.12 ± 2.70	13.33 ± 3.79	13.64 ± 3.06
	11.36 ± 2.74	9.25 ± 2.91	16.42 ± 3.67	12.62 ± 1.08
	15.65 ± 3.40	8.97 ± 2.56	13.47 ± 4.06	7.28 ± 0.93
Yellow	19.66 ± 2.71	15.17 ± 1.23	12.33 ± 1.07	18.73 ± 1.34
	9.24 ± 3.01	10.42 ± 2.57	14.42 ± 1.06	16.61 ± 4.27
	10.74 ± 2.45	15.48 ± 4.02	13.34 ± 0.38	16.12 ± 1.19
Scarlet	16.74 ± 3.01	16.78 ± 4.12	12.32 ± 0.83	13.95 ± 2.81
	11.91 ± 3.18	13.06 ± 0.43	12.78 ± 2.27	12.10 ± 1.08
	12.25 ± 1.09	9.71 ± 3.14	10.84 ± 2.56	9.76 ± 2.44

The three entries in each cell of the table are locomotion on agar, Drosophila culture medium and agar covered with a film of yeast, respectively. For each substrate, the set of sixteen matings was duplicated (N = 25 individuals per cell per replicate). Each entry in the table is the mean distance made by each larva tested in 2 min from the two sets of larvae measured (N = 50)

Table 2 Magnitudes of the genotype-environment interaction values (I_{strain}) and the magnitudes of environmental effects on locomotion of larvae of 48 and 96 h of *D. melanogaster*

Strain and genotype × environment interaction	Environment and larval age (h)					
	Agar		Drosophila medium		Agar plus yeast	
	48 h	96 h	48 h	96 h	48 h	96 h
$I_{OregonR-c}$	1.12	−3.55	−0.11	−5.90	0.09	2.42
I_{brown}	−0.69	2.85	1.14	−4.36	0.43	−1.51
I_{yellow}	−1.23	−2.18	1.05	3.57	0.17	3.62
$I_{scarlet}$	0.78	4.85	1.43	2.66	0.63	−2.55
Environmental effects	−0.75	1.16	0.34	−0.50	0.43	−0.63

Larvae tested belonged to the Oregon R–c, brown, yellow and scarlet strains (N = 50 individuals per strain and age)

Because larval locomotion of the Oregon R–c, *yellow*, *brown* and *scarlet* strains and their reciprocal hybrids was influenced by the substrate, we estimated magnitudes of genotype × environment interactions and environmental effects on locomotion at 48 and 96 h of larval age. We applied a linear function of four components: (i) mean character value of the four strains, (ii) expected phenotype of each of the strains, (iii) the average environmental effects, and (iv) the interaction effects (Lynch and Walsh 1998). The results are given in Table 2. At 48 h of larval development magnitudes of the genotype × environment interactions were similar to those of the environment effects. However, at 96 h of larval age the interaction effects were much greater than the magnitudes of the environmental effects, indicating

strong genotype × environment interactions (Table 2). These findings are in line with our contention that locomotion of 96 h-old larvae is more sensitive than that of 48 h-old larvae to differences in physical features of substrates.

Strictly speaking the strains used to perform the present diallel cross are not inbred. Thus, we examined the relationship between the variance (V_r) and parent-offspring covariance (W_r) of members of the same array (Mather and Jinks 1971). This is necessary for conformity of the data to the additive-dominance model of those authors and Hayman (1954). The results are shown in Fig. 1. On agar and *Drosophila* medium at 48 and 96 h of larval development, the points fell on a straight regression line. However, on agar covered with a film of yeast, the points are more scattered around the straight regression line, specifically for 48 h-old larvae (Fig. 1).

We performed a regression analysis of W_r on V_r for a 4 × 4 diallel in larvae of 48 and 96 h of development (Table 3). The corresponding correlation coefficients are close to one, with the exception of 48 h larvae on agar covered with yeast (r = 0.35). Regression analysis yielded slopes that are also close to one, with the exception of larvae of 48 h in the presence of food (slope equal to 0.04), and 96 h larvae on *Drosophila* medium (slope equal to 0.51). These results confirm that magnitude of changes in locomotion depends on larval age and the substrate on which the larvae moved. The functional relationship of W_r on V_r was also statistically significant (ANOVA, $p < 0.05$). In addition, r^2 values were all over 90.00%, with the exception of 48-h-old larvae on agar covered with yeast (2.37%). Furthermore, the Durbin-Watson test (Zar, 1984) showed that there was no serial autocorrelation in residuals at 95% of confidence level.

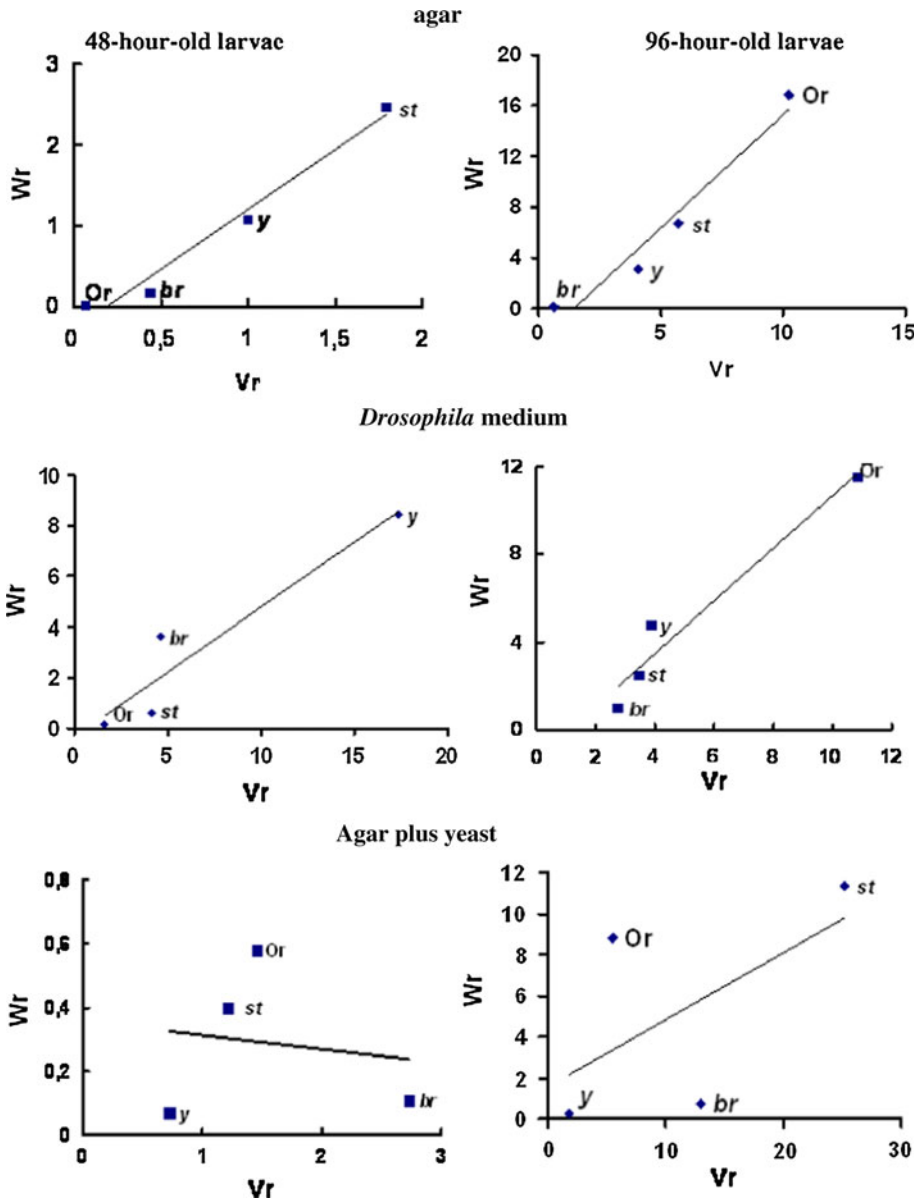

Fig. 1 The W_r/V_r graph for locomotion of larvae of 48 and 96 h of age in the diallel among the Oregon R–c (Or), brown (br), scarlet (st) and yellow (y) strains in *D. melanogaster* (data in Table 1). The slopes of the regression lines constitute Table 3

Table 3 Regression analysis of Wr on Vr for a 4 × 4 diallel in larvae of 48 and 96 h of development of the Oregon R–c, brown, yellow and scarlet strains of *D. melanogaster*

Item	Substrate and larval age (h)					
	Agar		Drosophila medium		Agar plus yeast	
	48	96	48	96	48	96
Correlation coefficient	0.99 ± 0.01	0.98 ± 0.02	0.98 ± 0.02	0.95 ± 0.03	0.35 ± 0.12	0.98 ± 0.02
Slope	1.49 ± 0.17	1.78 ± 0.28	1.17 ± 0.15	0.51 ± 0.12	−0.04 ± 0.20	1.78 ± 0.28
Intercept	−0.28 ± 0.18	−2.51 ± 1.72	−0.97 ± 0.91	−0.35 ± 0.06	0.36 ± 0.34	−2.51 ± 1.72

Altogether, these results suggest that an additive-dominance model describes our data well.

Regression of W_r on V_r (Fig. 1 and Table 3) shows negative intercepts, with exception of larval locomotion at 48 h in the presence of food. These findings suggest that F_1 hybrid larvae exhibit overdominance for this behavior. An examination of data in Table 1 shows that overdominance result in either increased or decreased locomotion depending on the substrate on which the larvae move.

It is also interesting to note that parental strains have different distributional patterns along the W_r on V_r straight line depending on the substrate and larval age (Fig. 1). Thus, at 48 h of age, on agar, the Oregon R–c strain is at the lower end of the regression line indicating that is a completely dominant parental strain. In this same environment, the *scarlet* strain is at the upper end of the regression line suggesting a completely recessive parent strain. However, at 96 h of development the Oregon R–c strain is a completely recessive parent, while the *scarlet* strain expresses some dominant alleles (Fig. 1). Again, these results suggest strong genotype × environment interactions for locomotion of *D. melanogaster* larvae.

Table 4 shows the mean squares and degrees of freedom for the analysis of variance of the 4 × 4 diallel (data in Table 1). At 48 and 96 h of larval development there is not substantial additive variation over the environments tested (item a). However, important dominance variation is present (b_3). The b item (dominance) and its portions b_1 (directional dominance) and b_2 (asymmetry of gene distribution) are not significant, but the b_3-residual dominance (dominance effects not attributable to b_1 and b_2)—item is significant. This discrepancy for b_3 could be a result of the fact that at 48 and 96 h of larval age some crosses show: (i) dominance to increase locomotion, (ii) dominance for decrease this behavior, (iii) no dominance for locomotion, (iv) overdominance to increase locomotion, and (v) overdominance to decrease locomotion, depending on the type

Table 4 Analysis of variance of the diallel data in Table 1

Source	df	MS			F		
		Agar	Agar + yeast	Medium	Agar	Agar + yeast	Medium
48-h-old larvae							
a	3	0.17	1.29	5.56	0.09	0.36	0.85
b	6	0.29	0.62	9.28	0.16	0.18	1.42
b1	1	2.86	2.86	2.55	1.60	0.82	0.39
b2	3	1.29	0.42	4.07	0.72	0.12	0.62
b3	2	781.50	888.35	478.79	433.89*	259.95*	73.43*
c	3	0.60	1.29	3.78	0.33	0.36	0.57
d	3	1.80	3.54	6.52			
96-h-old larvae							
a	3	0.30	10.00	8.06	0.07	0.67	0.50
b	6	5.69	9.53	2.61	1.28	0.64	0.16
b1	1	10.78	1.74	0.09	2.43	0.12	0.005
b2	3	9.92	5.05	0.52	0.75	0.34	0.03
b3	2	3760.08	1568.57	1886.03	141.46	105.84	116.71*
c	3	23.93	4.21	5.14	1.80	0.28	0.32
d	3	13.28	16.16				

* $P < 0.05$

of substrate. An examination of Table 1 suggests that this diversity of responses in the F_1 is greater at 96 than 48 h of larval age. In summary, the results from the diallel cross showed that at 48 and 96 h of larval age there is dominance genetic variation. These results are in agreement with those shown in Fig. 1.

Discussion

In this work we investigated locomotion of *D. melanogaster* larvae by examining changes in this behavior as larval development proceeds, and in response to environmental modifications. We found that *D. melanogaster* larval locomotion increased over time and demonstrated plasticity in response to different environments. This behavioral flexibility was observed in larvae of both ages of development that we tested, but the response was stronger for 96 h old larvae than 48 h old larvae. We also found that, under experimental conditions used, dominant genes control locomotion of *D. melanogaster* larvae, thus confirming the findings of Godoy-Herrera et al. 1984. This type of genetic architecture is typical of traits linked to biological fitness (Falconer and Mackay 1996).

We have shown that the magnitude of genotype × environment interactions on locomotion of *D. melanogaster* larvae changes with larval age. While variation in locomotion between young larvae of the same strain appears to be similar in magnitude to environmental effects and the genotype × environment interactions, the effect of genotype × environment interactions on variance is clearly more important for older larvae. In view of these findings, it is not surprising that the *Wr* on *Vr* graphs (Fig. 1) show different ordering of the strains. Additionally, the graphs provide some evidence of dominance, though it would not be totally correct to conclude that the strains differ reliably in their dominance properties for this measure. Nevertheless, the analysis of variance of the diallel table provides tests of significance for the heritable components of variation. In this case the information from the graphs (Fig. 1) and diallel tables obtained for larval locomotion at 48 and 96 h of larval development suggests a strong dominant component.

Age-related phenotypic changes in locomotion make sense because larvae of different ages use locomotion for different purposes. While young larvae employ locomotion to locate food sources within a breeding site unit, old larvae use locomotion to select a substrates on which to pupate (Medina-Muñoz and Godoy-Herrera 2004). Thus, in natural breeding sites searching for food and pupation sites are main activities of the larvae. Consequently, these age-related changes in locomotion can be interpreted as mechanisms of adaptation to the ecology of their spatially and temporally heterogeneous breeding sites.

More specifically, *Drosophila* breeding sites include the yeasts and bacteria that initiate the decaying process and also provide proteins, sterols and vitamins to developing larvae (Starmer and Aberdeen 1990). In the wild, the process of decay is heterogeneous even within a particular breeding site. For example, yeast growth on a variety of fruits as apples and prickly pears in Central Valley of Chile is patchy. As the original colonies of bacteria and yeasts are consumed by larvae, new colonies of microorganisms rapidly appear (unpublished data). Later in development, *Drosophila* larvae employ locomotion to select pupation sites. In nature, larvae pupate in a variety of microhabitats which differ in compaction, humidity and texture. We have found *D. melanogaster* pupae over and under the skin of decaying grapes, on the ground underneath the fruit, and away from the grapes (an average of 3.0 ± 0.94 cm away). Variation in physical and chemical characteristics of *Drosophila* breeding sites, and differences in microhabitats that larvae use for pupation provide frameworks for understanding the role of environment in larval locomotion.

Our results also demonstrate a genetic component to phenotypic variance in locomotion. Depending on the cross, dominance and overdominance effects resulted in either an increase or decrease in locomotion. Because we did not use inbred lines, the diversity of responses for locomotion in the F_1 could reflect genetic segregation for the behavior. Genetic variation in locomotion of *D. melanogaster* larvae could be especially important when larvae of other *Drosophila* species are present in their same breeding site. In this case, specific strategies of foraging and pupation site selection could allow for the coexistence of species with a similar ecology within the same breeding site (Medina-Muñoz and Godoy-Herrera 2004; Sokolowski et al. 1986).

A number of *Drosophila* species develop in changing, heterogeneous environments with features such as pH, temperature, sugar content and humidity varying over time (David et al. 1983). The decaying process liberates a variety of substances that further contribute to environmental heterogeneity, and which larvae of different *Drosophila* species must also adapt (Starmer et al. 1977). Our data show that *D. melanogaster* of 48 and 96 h old larvae use locomotion flexibly depending on the type of substrate. These characteristics of locomotion are likely a result of adaptive evolution of *Drosophila* larval behavior in response to the variability of natural breeding sites. In order to fully understand the role of genotype × environment interactions in larval locomotion, we also need to investigate how locomotion interacts with feeding, turning, and several other larval behaviors to construct higher order behavioral traits, including foraging and pupation behavior

(Green et al. 1983; Godoy-Herrera and Connolly 2007; Beltramí et al. 2010). These higher order behavioral traits require flexibility in larval behavior in order to facilitate exploration and inspection of environment while searching for food and pupation sites, and escaping from predators, e.g., ants. In fact, plasticity of locomotion could be a key for survival of *D. melanogaster* which breeds in a variety of decaying substrates and different climates around the world.

We conclude that phenotypic flexibility and variability in locomotion shown by *D. melanogaster* larvae makes sense in the context of development and habitat selection. Our data suggest that flexibility and plasticity of *D. melanogaster* larval locomotion depends upon a combination of the physical features of the environment, the distribution of food sources within the breeding sites, and genetic determination of this behavior.

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