Genetics Analysis of Larval Foraging Behavior in *Drosophila Funebris*

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Abstract To understand the genetics and evolution of foraging in larvae of *Drosophila funebris*, we examined two strains reared at different breeding sites in the wild. Larvae of the Til–Til strain breed in necrotic cactus tissue, while those of the Pelequén strain rear in necrotic prickly pear cladodes. We measured feeding, locomotion, turning behavior, and latency of *D. funebris*. Til–Til and Pelequén larvae, at 8 days of age show very similar rates in all behaviors. Crosses between Til–Til and Pelequén strains decrease feeding rate and increase locomotion, turning, and latency in F_1 and F_2 larvae. Backcross larvae show a behavior similar to that of their parental strains. The behavioral similarities observed between the Til–Til and Pelequén strains are product of two different co-adapted gene pools. Epistasis and dominance are the principal sources upon which adaptation of the gene pools of each population are based.

Keywords *Drosophila funebris* larval foraging · Genetic co-adaptation · Epistasis · Dominance

Drosophila larvae feed and pupate in changing environments due to microorganisms that cause fermentation within breeding sites (David et al. 1983; Brncic 1987a; Fogleman et al. 1981; Fogleman and Abril 1990). The fermentation process produces toxic substances that contaminate the rearing sites in a short time (Chakir et al. 1996; Bochdanovits and De Jong 2003). *Drosophila* breeding sites also have a central role in the maintenance of genetic variation related with larval food acquisition and the metabolism of ethanol (Osborne et al. 1997; Pecsenye et al. 2004); and in the resource partitioning by *Drosophila* species with similar ecology (Medina-Muñoz and Godoy-Herrera 2004; Mery and Kawecki 2004). Changing environments at variable *Drosophila* breeding sites favor larval learning and habitat selection (Davis and Stamps 2004). So, natural rearing environments of *Drosophila* larvae are requisite to any understanding of population genetics and evolution of this genus (Carson 1971).

The rearing sites of many *Drosophila* species also affect the adult phase of the life cycle. For example, they play a critical role in the evolution of reproductive strategies of many species in this genus (Kambysellis and Heed 1971; Powell 1997; Markow and O'Grady 2006). *Drosophila* breeding sites also influence the selection of oviposition sites (Barker and Starmer 1999; Mery and Kawecki 2004).

In this work, we propose that in *Drosophila* the genetics and evolution of behavioral components of larval foraging, as feeding, locomotion, and turning are subject to selective pressures, as a product of changing stressful conditions imposed by fermentation processes and by the presence of larvae of other *Drosophila* species within breeding sites.

To test this hypothesis we examined two natural populations of *D. funebris* that breed in different sites in the wild. Larvae of the Til–Til strain develop in necrotic cactus *Echinopsis chilensis*; larvae of no other *Drosophila* species breed on this substrate (Manríquez and Benado 1994). Larvae of the Pelequén strain breed in necrotic prickly pear cladodes (*Opuntia ficus-indica*) together with *Drosophila*

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buzattii and *Drosophila simulans* (Flores 2004). We tested genetically variable strains from each population to examine the possible effects of hybridization of genetically unrelated individuals on the expression of behavioral components of larval foraging. *Drosophila* larval behaviors are sensitive to environmental variations (Muhammad-Ali and Burnet 1995). Thus, the natural populations examined could show different expressions of the same larval behaviors. We also measured latency of foraging, an indicator of adaptation to new environments (Martin and Bateson 1990).

Cosmopolitan *D. funebris* belongs to the *funebris* group comprising eight species in the subgenus *Drosophila* (Markow and O'Grady 2006).

Materials and methods

Subjects

Drosophila funebris were collected in Til–Til from necrotic cactus *Echinopsis chilensis*. Til–Til is located 50 km Northwest from Santiago; the annual rainfall is about 180 mm. In the hills surrounding Til–Til plants of *E. chilensis* grow with other native vegetation. We also formed another *D. funebris* strain with adults, which emerged from decaying cladodes of *Opuntia ficus-indica* collected in Pelequén, 100 Km South of Santiago, where the annual rainfall is about 290 mm. Adults of *D. funebris*, *D. buzzatii*, and *D. simulans* emerged from the cladodes. We deliberately established genetically variable strains of *D. funebris*. Thus, we founded each wild isolate with approximately 25 flies (Til–Til, TT, and Pelequén, P, strains), and the sex ratio was variable. When we made the crosses, four generations of breeding in the laboratory had elapsed (see below). This procedure ensured that nearly all of the genetic variation present in the founders was retained when the strains were crossed.

Flies were all reared under constant light at 18 ± 1°C in 250 cc glass bottles in Burdick's medium (Burdick 1954). Facilities to change the Light / Dark cycle were not available in the laboratory. All experimental flies were raised and stored under the same conditions.

Collection of eggs and larvae

Groups of 60–70 inseminated females of the TT and P strains, and the hybrids between these strains were allowed to lay eggs for 3–4 h on their respective plastic spoons filled with Burdick's medium (Burdick 1954). We measured the behavior of larvae at eight ages. We collected larvae in 4-h windows every day for 8 days, at 1–8 days after hatching. Thus, we examined the behavior of 1-day-old through 8-day-old larvae. Medium in the spoons was supplemented daily with a drop of 48% fresh baker's yeast (principally *Saccharomyces cerevsiae*) paste.

Crosses

We examined the genetics of feeding, locomotion, and turning behavior of *D. funebris* larvae (TT and P strains). We deposited larvae individually onto the center of agar Petri dishes. We previously overlaid the agar with a film of 48 % of yeast paste. We tested each individual in a fresh Petri dish for two minutes. We observed fifty larvae of each age, and generation. Feeding rates were calculated as numbers of scooping movements per minute by mouth hooks and cephalopharyngeal apparatus. Locomotion equaled the numbers of waves of segmental contraction per minute passing in series along the body. We estimated turning behavior as numbers of turns per minute as larvae crawl on substrates (Green et al. 1983). We also recorded the time elapsed from the moment a larva was deposited in the Petri dish until it began to respond (latency).

We crossed the TT and P strains reciprocally. We tested larvae of 1-day-old up to 8-day-old of the parental strains, F_1's, F_2's and backcrosses (see Table 1). Here we reported principally foraging behavior and latency in 8-day-old larvae. When age effects are important, we mention in the text.

For the valid application of the model of Mather and Jinks (1971) the assumption of additivity needs to tested. So, we tried to remove multiplicative effects by changing scale. We transformed the data to logarithms; this scale is used to convert multiplicative into additive effects (Sokal and Rohlf 1995). However, transforming to logs did not remove multiplicative effects. We followed the analysis applying scaling tests to examine the adequacy of the results in an additive-dominance model (Mather and Jinks 1971, Kearsey and Pooni 1996). These tests consider the relationships between the generation means. Using this transformed data, we estimated the additive, dominance, and epistasis parameters concerning feeding, locomotion, turning, and latency.

We also used ANOVA to compare the parental and F_1 generations. We tested whether the parental strains were different from each other, whether there were reciprocal differences in the F_1's, and whether the F_1's showed dominance. To determine whether there were maternal or sex-linkage effects we performed an analysis of variance of all 10 crosses (Table 1). Thus, we compared TT and P parental strains, F_1's, F_2's and reciprocal backcrosses. For example, we tested whether larvae of backcross F_1 males to TT females were different in foraging from ones of the reciprocal cross of F_1 females to TT males.

Table 1 Means and variances for larval feeding rate, locomotion, turning, and latency obtained to cross the Til–Til, TT, and Pelequén, P, strains of *Drosophila funebris*. Larvae tested were 8 days old

Cross	Feeding rate (counts/min)	Locomotion (body contractions/min)	Turning (turns/min)	Latency (s)
Parentals				
TT × TT	2.09 (0.13)	1.30 (0.14)	0.71 (0.12)	0.71 (0.34)
P × P	2.14 (0.14)	1.29 (0.11)	0.68 (0.09)	0.72 (0.41)
F_1				
TT × P	1.38 (0.22)	2.31 (0.12)	1.07 (0.28)	1.17 (0.97)
P × TT	1.35 (0.24)	2.29 (0.14)	1.08 (0.19)	1.14 (0.92)
Backcrosses				
TTP × TT	2.09 (0.13)	1.29 (0.20)	0.72 (0–12)	0.83 (0.42)
TT × TTP	2.19 (0.10)	1.30 (0.16)	0.81 (0.11)	0.68 (0.45)
PTT × P	2.14 (0.12)	1.29 (0.17)	0.74 (0.13)	0.76 (0.52)
P × PTT	2.19 (0.11)	1.29 (0.13)	0.73 (0.09)	0.87 (0.34)
F_2				
TTP × TTP	1.13 (0.28)	2.28 (0.18)	1.65 (0.71)	1.48 (0.85)
PTT × PTT	1.29 (0.24)	2.29 (0.21)	1.69 (0.79)	1.44 (0.93)

For all crosses, the first parent shown is the female. Data transformed into logarithms

Results

Table 1 shows the means and variances for larval feeding rate, locomotion, turning, and latency. Larvae were 8 days old. The pattern of inheritance for these components of foraging, and for latency in larvae of *D. funebris* does not follow that of additive polygenic model (see also Fig. 1). For example, larvae of the parental strains, TT and P, and the four backcrosses show a similar feeding behavior (Table 1 and Fig. 1). Moreover, the F_1's and F_2's exhibit larval feeding rates lower than that of the parental and backcross generations (ANOVA, $F_{9,491} = 15.20$, $P < 0.05$). Thus, though the cross between TT and P strains decreases feeding rate in larvae of the F_1 and F_2 generations, incorporation of genes from parental lines into the F_1's restores the high feeding rates observed in TT and P strains. Larvae at 1, 2, 3, 4, 5, 6, and 7 days of age also showed a pattern of inheritance for feeding rate similar to that of 8-day-old larvae.

In contrast to feeding, reciprocal crosses between TT and P strains increase locomotion and turning, and latency period in 8-day-old larvae of F_1 and F_2 (Table 1). Incorporation of genes from parental lines into the F_1's restores in backcross larvae a behavior similar to that observed in TT and P strains (ANOVA, $F_{9,491}$: 14.10, 24.36, 11.69 for locomotion, turning rate, latency, respectively, $P < 0.05$. This also suggests patterns of inheritance for locomotion, turning and latency that do not agree with an additive polygenic model.

Hybridization also affected locomotion, turning rate, and latency in larvae of 1, 2, 3, 4, 5, 6, and 7 days old of the F_1's and F_2's. The larvae moved less than 8-days-old larvae, rate of turning fluctuated through larval development, and latency increased. However, larvae of 1, 2, 3, 4, 5, 6, and 7 days of the backcrosses exhibited a locomotion, turning rate, and latency similar to that of the parental lines.

The two F_1's did not show a maternal influence on feeding, locomotion, turning, and latency. The phenotypes of the F_1's were not significantly different from each other (ANOVA, $F_{7,464} < 2.05$, ns). This same analysis also showed that differences between the four reciprocal backcrosses, and the parental strains, TT and P, for the foraging components, and duration of latency were not significant (ANOVA, $F_{21,960} < 1.67$, ns).The mean phenotypes of the four backcrosses and the TT and P parental strains are similar. Behavior of larvae of the two F_2 generations was also very similar (ANOVA, $F_{7,464} = 1.98$ ns). We conclude that maternal and sex-linkage effects on foraging, and latency are not significant in 8-days-old larvae of *D. funebris*. The behavior of larvae aged 1, 2, 3, 4, 5, 6, and 7 days old of all 10 crosses was similar to that of 8-day-old larvae.

The log transformation equalized the variances for components of foraging, and latency in 8-day-old larvae of the parental and backcross generations (Table 1). A F test for differences between two variances yielded values lower than critical value ($F_{49,49} < 1.75$; ns). The F_1 and F_2 generations still showed greater variances than the parental and backcross generations. F values for differences between variances were over than critical value ($F_{49,49} > 1.75$; $P \ll 0.05$). These results also suggest that patterns of inheritance for feeding, locomotion, turning, and latency deviate from an additive polygenic model. Log transformation also yielded similar results for the variances of larvae of 1, 2, 3, 4, 5, 6, and 7 days of age.

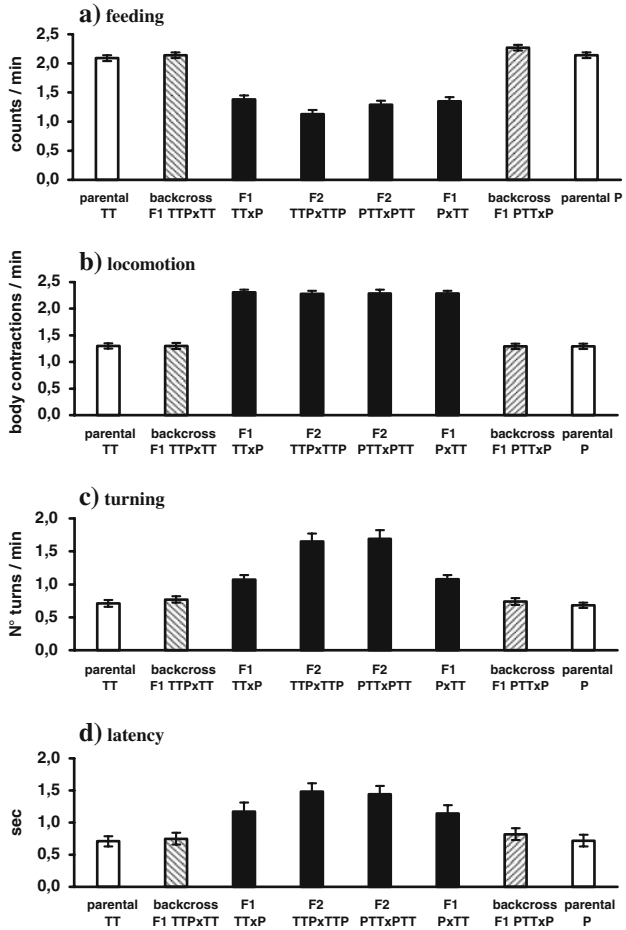

Fig. 1 (**a**) Feeding, (**b**) locomotion, (**c**) turning, and (**d**) latency (mean ± standard error) of larvae of the Til–Til (TT) and Pelequén (P) *Drosophila funebris* populations (white columns), and their derived F_1, backcross, and F_2 generations. Reciprocal F_1 and F_2 are represented by black columns (see Table 1). Shading columns correspond to backcrosses F_1 TTP × TT and F_1 PTT × P. Each backcross is mean of the corresponding reciprocal backcrosses (see Table 1). Rate of feeding are expressed as counts per minute. Locomotion is expressed as larval body contractions per min. Turning behavior is estimated as numbers of turns per min. Latency is expressed in seconds

Scaling tests

Scaling tests (A, B, C) yielded figures that were not compatible with a simple additive-dominance model (t tests produced values over the critical value of 2.06, df = 24). In addition, the χ^2 test measuring goodness of fit to the additive-dominance model (the joint scaling test) also produced the same results as the t test. Thus, scaling tests also suggest that epistasis is a principal source in the control feeding, locomotion, turning, and latency in 8-day-old larvae. The same analysis applied to these same behaviors of larvae of 1, 2, 3, 4, 5, 6, and 7 days of age also produced similar results. That is, through larval development, epistasis is involved in the control of foraging behavior of TT and P *D. funebris* strains.

Estimation of genetic parameters

Table 2 shows an estimation of the additivity, dominance, and epistasis for feeding, locomotion, turning, and latency. Larvae were 8 days old. The results again indicate that in the larvae of TT and P *D. funebris* strains dominance and epistasis control foraging behavior and latency components [*d*], [aa] and [*dd*]. Epistasis occurs principally between dominant genes, [*dd*]; some significant epistasis between additive genes also exists, component [*aa*]. These estimates are in good agreement with data presented in Table 1, the scaling tests, and Fig. 1. Foraging behavior and latency in larvae 1, 2, 3, 4, 5, 6, and 7 days of age from the TT and P strains also showed significant dominance and epistasis.

Discussion

Drosophila funebris larval foraging

This work demonstrates that the Til–Til, TT, and Pelequén, P, strains of *D. funebris*, reared in different breeding sites in the wild, have similar phenotypic means for feeding, locomotion, and turning. These behavioral similarities are, however, the product of two very different genetic architectures. In fact, genetic analysis of these behaviors strongly suggests that two different co-adapted complexes underlying larval foraging have evolved in the two *D. funebris* strains. Genetic analysis also indicates that epistasis and dominance are the principal sources that generate cohesion in the gene pools of the TT and P strains. The two strains of *D. funebris* showed little change of behavior with larval development, except some minor fluctuations in some of the behaviors exhibited by larvae of the F_1's and F_2's. Genetic introgression of parental strains into the F_1's restores in backcross larvae a behavior similar to that observed in TT and P larvae. These results were hardly unexpected. We deliberately established genetically variable strains. We crossed these strains after four generations in the laboratory. This procedure ensured that a substantial amount of founder genetic variation remained (Arizmendi 2004).

The results reported here suggest that natural selection could be an evolutionary force leading to the differences in larval foraging behavior between the TT and P *D. funebris* strains. Til–Til larvae breed in the columnar cactus *Echinopsis chilensis*, while larvae of the P strain rear in *Opuntia ficus-indica*. Fresh tissues of these cacti differ in chemical composition, particularly in sugars; concentration of volatile alcohols in necrotic tissue of these cacti is

Table 2 Estimation of the additive, dominance and epistasis parameters for the feeding, locomotion, turning, and latency in *Drosophila funebris* larvae

Behavior	Parameter					
	[*m*]	[*a*]	[*d*]	[*aa*]	[*ad*]	[*dd*]
Feeding	3.22 ± 0.76	0.01 ± 0.01	2.48 ± 0.22*	0.80 ± 0.02	0.02 ± 0.0	11.00 ± 0.78*
Locomotion	4.85 ± 0.68	–	9.89 ± 0.23*	4.59 ± 0.01*	0.11 ± 0.01	6.90 ± 0.03*
Turning	2.24 ± 0.34	0.01 ± 0.01	4.10 ± 1.14	2.16 ± 0.04*	0.02 ± 0.01	2.42 ± 0.26*
Latency	4.13 ± 0.57	0.01 ± 0.01	9,25 ± 2.16*	4.06 ± 0.57*	0.18 ± 0.01	6.87 ± 0.68*

Means used are in Table 1. Larvae were 8 days old. For all crosses the parental lines were the Til–Til and Pelequén strains. [*m*] = Common effects to every genotype; [*a*] = Additive component; [*d*] = Dominance effect of means: [*aa*] = Additive × additive interaction; [*ad*] = Additive × dominance interaction; [*dd*] = Dominance × dominance interaction

* $P < 0.05$

different (Starmer 1981; Fogleman and Danielson 2001). P larvae of *D. funebris* coexist with larvae of *D. buzzatii* and *D. simulans* in cladodes of prickly pear (Flores 2004), whereas TT larvae breed in *E. chilensis* in the absence of larvae of other *Drosophila* species (Manríquez and Benado 1994). Thus, there are important ecological differences between the breeding sites used by the TT and P *D. funebris* strains. Natural selection could act on the gene pool of each population to modulate the expression of behavioral elements that constitute larval foraging, particularly feeding rate. A high feeding rate could excess cellular capacity of detoxification (Fogleman and Danielson 2001). In contrast to the findings reported here, larvae of Chilean natural populations of endemic *D. pavani* and cosmopolitan *D. melanogaster* and *D. simulans* bred in apples, grapes, and peaches increase feeding rates, locomotion and turning as larval development proceeds (Ruiz-Dubreuil et al. 1996; Godoy-Herrera et al. 2004, 2005; Medina-Muñoz and Godoy-Herrera 2004).

Population genetics of *D. funebris* larval foraging

Our data show that hybridization decreases feeding rates in larvae of the F_1 and F_2 generations, but it increases locomotion, turning, and latency (Table 1 and Fig. 1), as larvae of the F_1 and F_2 generations between TT and P strains of *D. funebris* show negative heterosis for feeding rate and positive heterosis for locomotion and turning. Crosses between TT and P strains also disrupted capacity of larvae to adapt to new environments, as suggested by the increase in latency.

Our results also support the contention that the TT and P *D. funebris* strains differ in co-adapted gene pools controlling foraging. Co-adaptation implies the organization in natural populations of specific interactions of genes that maximize fitness (Wallace 2000). One kind of evidence for co-adaptation is that combinations of genes derived from different populations alter the expression of one or more traits related to fitness. Our data provide substantial proof of evolutionary divergence for larval foraging behavior between gene pools of the two *D. funebris* strains examined here.

Foraging depends on coordination and integration between feeding, locomotion, and turning (Godoy-Herrera and Connoll 2007). We observed increases in the latency to restart locomotion, feeding, and turning after disturbance produced by handling in the F_1's and F_2's hybrid larvae. We also noted that the hybrid larvae had a high incidence of failure to re-establish ventral body contact with the substrate. For example, several larvae of the F_1's and F_2's intended to crawl on one side making sluggish scooping movements by the mouth hooks. These all may be indications of generalized impairment of motor control produced by hybridization of gene pools. These results also indicate that the two *D. funebris* strains studied in this work have genetically diverged in larval foraging and capacity to adapt to new environments.

Our results indicate evolutionary importance to relate foraging behavior of *Drosophila* larvae with fermentation processes that occur within the breeding sites, and the presence of larvae of other species of *Drosophila*. Larvae of the Til–Til and Pelequén *D. funebris* strains show similar foraging rates and latency. That is, in the two strains quantitative expression of these behaviors have not changed. However, genetic divergence in the control of foraging, and behavioral latency has taken place. We would like to suggest that the ecology of *Drosophila* breeding sites provides valuable information on past and current evolutionary forces affecting larval behavioral traits, and so genetic structures of populations of *Drosophila* species.

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