The development and genetics of digging behaviour in *Drosophila* larvae

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Larval digging behaviour of *Drosophila melanogaster* and the sibling species *Drosophila pavani* and *Drosophila gaucha* differs quantitatively. Digging activity increases with age. Crosses between four strains of *D. melanogaster* indicate dominance and heterosis for digging; some non-allelic interaction is also suggested. Larval locomotion of a line with low digging behaviour is lower than that exhibited by larvae of a line with high digging behaviour. Larvae of the low digging line form puparia away from the medium, while those of the high digging line select places close to the substrate. However, larvae of a separate digger strain (the Oregon R–C strain) show similar locomotor behaviour to that of larvae of another non-digger strain (the *vestigial* strain). It is suggested that, besides locomotion, photoreponse of larvae may also influence the dispersal pattern within food. Dominance and heterosis towards high digging suggest possible evolutionary importance for this behaviour.

INTRODUCTION

Most animal species show a characteristic pattern of dispersal in the wild (Brown, 1975). Among *Drosophila* species, dispersal patterns of adult flies have been reported for factors such as food, reproduction and conspecifics (Spieth, 1974; Grossfield, 1978; Godoy-Herrera and Fenner, 1984). Few reports have been published on larval dispersal patterns in *Drosophila*, although burrowing is a behaviour common to a variety of *Drosophila* species. McCoy (1962) has found that with the exception of *Drosophila busckii*, larvae of *Drosophila melanogaster*, *Drosophila hydei*, *Drosophila tripunctata* and *Drosophila quinaria*, dig vigorously within overripe fruits of tomatoes.

MacArthur and Levins (1967) and Pianka (1982), have argued that natural selection favours organisms that specialise on a limited portion of the available resources which they exploit more efficiently than their competitors. In *Drosophila*, larvae of many species share similar nutritional requirements, one example being the sibling species *D. melanogaster* and *Drosophila simulans* (review in Ehrman and Parsons, 1981). Here I want to propose that larval digging behaviour of *Drosophila* is an adaptation to distribute themselves in space allowing optimal utilisation of

resources such as food and pupariation places, preventing competition for these resources. Burrowing behaviour of *D. melanogaster* larvae seems to have a strong genetic component that persists under laboratory conditions (Godoy-Herrera, 1977; 1978).

Larvae of *Drosophila* also show other behavioural responses, all of which may be connected with feeding and pupariation. Green *et al.* (1983) provide a description of the organisation of larval behavioural patterns in *D. melanogaster* and *D. simulans*. Larvae of *D. melanogaster* ingest food with a variable rate depending on age and genetic background (Sewell *et al.*, 1975). Larvae of this species also show foraging responses (Sokolowski, 1980). All these behaviours are poligenically controlled (Burnet *et al.*, 1977; Sokolowski, *op. cit.*; Sewell *et al.*, *op. cit.*, 1975). Besides, Godoy-Herrera *et al.* (1984) have found that young larvae of *D. melanogaster* show a preference for moist substrates, while mature larvae prefer a dry substrate on which to crawl. These authors have also shown that, in *D. melanogaster*, increase in larval locomotion with age is caused by growth in body size and changes in the genetic architecture of this behaviour. Sokolowski *et al.* (1984) have found that larvae of *D. melanogaster* of 3 to 4-days-old stay on food, but larvae at

5-days-old tend to leave food for a more dry place; these behaviours are expressed sequentially during the larval development of this species.

The aims of this paper are to describe burrowing behaviour of larvae of *D. melanogaster* and the sibling species *Drosophila pavani* and *Drosophila gaucha*, and to provide a more detailed genetic analysis of digging behaviour by crossing four strains of *D. melanogaster* which show marked differences in digging (Godoy-Herrera, 1977).

MATERIALS AND METHODS

(i) Stocks

Drosophila pavani, Brncic, 1957. This species is endemic to Chile (Brncic, 1970). The strain used in this research was derived from wild flies collected in 1955 by Brncic (1957) at La Serena (Latitude 30°S), and maintained since then by mass culture in the laboratory.

Drosophila gaucha, Jaeger and Salzano, 1953. The flies used were of the "Tainhas" strain kindly supplied by the Department of Genetics, University of Rio Grande do Sul (Brazil). The strain is also kept by mass culture in the laboratory.

These two species are sibling and make up the *mesophragmatica* group together with another six species all morphologically very similar (for details see Brncic, 1970).

Drosophila melanogaster, Meigen, 1830. The following strains were employed in this work: Oregon R-C (wild type), *taxi* (*tx*), *yellow* (*y*), and *vestigial* (*vg*). Larvae of the high and low digging lines (F_{20} generation) derived from the Oregon R-C strain by selective breeding (Godoy-Herrera, 1978) were also used (see below). All species and strains used here were kept in half-pint bottles filled with about 50 cm³ of Burdick's medium (1954) at 24°C with a constant regime of light.

(ii) Vials with charcoal and egg collections

Vials 2.5 × 10 cm were filled with 4 ml of culture medium mixed with finely powdered charcoal (2 gm of charcoal/100 ml of medium). After coding, this medium was covered with 2 ml of the same medium without charcoal. Depth of culture medium without charcoal was about 4 mm.

The type of food used permits the recognition of two kinds of larvae hatched from the eggs laid over the food medium: (1) those that ingest medium with charcoal (named "Stained larvae" in the paper) and (2) those that eat medium without charcoal (denominated "Unstained larvae" in this

work). Larvae which have eaten food with charcoal show a stained digestive tract under a stereomicroscope; those that have not fed on this medium exhibit a transparent digestive tract. Most of mature stained larvae ready to pupariate have particles of charcoal in their digestive tract, as seen in larvae crawling on the walls of the shell vials. Larvae showing a transparent digestive tract have presumably stayed eating in the upper zone of the food medium (for other details on the method see Godoy-Herrera, 1977; 1982).

Groups of 60–80 inseminated females of the Oregon R-C, *taxi*, *yellow* and *vestigial* strains, and of the high and low digging lines were left without food for a 3 hour period in half-pint bottles. Starvation stimulates oviposition. After this period I introduced into each one of the bottles a plastic spoon filled with medium spread with fresh live yeast and acetic acid. These two substances stimulate oviposition. Eggs laid by females were collected after 3–4 hours with the aid of a dissecting needle. This was previously sterilised by flaming and cooling in alcohol to reduce the risk of contamination of the vials by fungi and/or bacteria.

(iii) Digging behaviour

One hundred and twenty vials with medium with charcoal (lower zone) and without charcoal (upper zone) were each sown with 30 eggs of the Oregon R-C strain. Similar sets of these same vials were also each sown with 30 eggs of the *taxi*, *yellow* and *vestigial* strains. Eggs were carefully deposited over the surface of the nutritive medium to avoid scars with the needle. Young larvae seem to begin their burrowing activities through scars and/or holes in the surface of the culture medium.

The development of larval digging behaviour of the Oregon R-C, *taxi*, *yellow* and *vestigial* strains was measured as the percentage of stained larvae at 60, 72, 84, 96, 108 and 120 hours of age. Very few larvae reach the lower zone before 60 hours. In each interval of time a set of 20 vials was examined. The same procedure was followed for studying digging behaviour of F_1 hybrid larvae produced by crossing the Oregon R-C, *taxi*, *yellow* and *vestigial* strains in all possible combinations. The arcsin transformation was used (Bartlett, 1947). Means of stained larvae of F_1 reciprocal crosses were statistically analysed for possible maternal effects or other kinds of reciprocal effects.

In the case of *D. pavani* and *D. gaucha*, mature 14–15-day-old females were used to collect eggs (Koref-Santibañez and del Solar, 1961). In these species the larval period at 24°C is longer than in

D. melanogaster (Brncic *et al.*, 1969). The number of stained and unstained larvae were recorded each 12 hours between 60 and 192 hours of development. In each interval of time 20 vials were examined. Each vial was sown with 30 eggs of *D. pavani* or *D. gaucha*. The development of digging behaviour in larvae of these two species was compared by taking the number of stained and unstained larvae at the different ages to calculate χ^2 in 2×2 contingency tables (Sokal and Rohlf, 1969).

(iv) Locomotor activity

A rectangular 7.5 × 14.5 cm piece of glass was covered with a layer of agar 3 mm thick. At 108 hours larvae of the Oregon R-C (digger strain), *vestigial* (non-digger strain) and the high and low digging lines were individually transferred onto the centre of the agar. Larvae used were reared in vials with medium without charcoal. Most of the larvae examined were picked off from the walls of the shell vials. After a settling-down period of 30 seconds larval locomotion was observed for 3 minutes and graphed using a camera lucida.

Length of the track made by each larva was measured by using a Hoffritz Map Measurer. Distance obtained for each track was converted into centimetres.

(v) Pupariation of larvae of the high and low digging lines

In each generation of selection (F_1 to F_{20}) for high and low digging behaviour (Godoy-Herrera, 1978), two half-pint bottles, filled with 50 cm³ of medium without charcoal, were each sown with 200 eggs of each one of these lines. Distance between the surface of the medium and each one of 120 pupae were recorded at 148 hours of development.

RESULTS

(i) Interspecific differences in digging

Fig. 1 shows the development of digging behaviour in larvae of *D. melanogaster*, *D. pavani* and *D. gaucha*. At 60 hours of development about 8 per cent of larvae of *D. melanogaster* have dug down

Figure 1 The development of digging behaviour in larvae of *D. melanogaster* (●), *D. pavani* (○), and *D. gaucha* (△) expressed as percentage of stained larvae at different larval ages (see Materials and Methods).

to the medium with charcoal, while most of the larvae of *D. pavani* and *D. gaucha* stay in the upper zone of the medium. Between 72 and 96 hours the percentage of *D. melanogaster* larvae that have reached medium with charcoal progressively increases up to about 92 per cent; for 120 hours only 3 per cent of *D. melanogaster* larvae exhibit a clear digestive tract without charcoal.

Fig. 1 shows that at 96 hours of development 18 per cent of larvae of *D. pavani* have reached the medium with charcoal. After 144 hours of age 100 per cent of larvae of this species have eaten medium with charcoal. In contrast, the percentage of *D. gaucha* larvae stained with charcoal only increases after 108 hours of development; at the end of the larval period (192 hours of age) about 90 per cent of larvae of *D. gaucha* have burrowed down to medium with charcoal.

D. melanogaster has a larval period shorter than the sibling species *D. pavani* and *D. gaucha*. Comparisons between digging behaviours of larvae of these three species are difficult. In table 1 only digging activities of larvae of *D. pavani* and *D. gaucha* are statistically compared. It can be seen that with the exception of larvae at 144 hours of development, the pattern of larval dispersal of *D. pavani* within the medium is different from that of *D. gaucha*. These findings suggest that during the trophic stage of development *D. pavani* larvae consume food in zones of the substrate much deeper than *D. gaucha* larvae.

(ii) Genetics of digging behaviour

Fig. 2 (a, b, c) shows the development of digging behaviour in hybrid larvae obtained by crossing

the two non-digger strains (the *vestigial* and *yellow* strains) with two digger strains (the Oregon R-C and *taxi* strains). Between 60 to 120 hours of development, F₁ hybrid larvae obtained by crossing the *vestigial* and Oregon R-C strains dig within the medium like their digger parents of the Oregon R-C strain (Fig. 2 (a)).

Fig. 2(b) shows that F₁ hybrid larvae obtained by crossing *vestigial* flies (non-digger strain) with *taxi* flies (digger strain) also burrow into the culture medium as their digger parents of the *taxi* strain. On the other hand, F₁ hybrid larvae obtained by crossing two non-digger strains (the *vestigial* and *yellow* strains) tunnel into the substrate in a proportion greater than larvae of the *yellow* strain, indicating hybrid vigour for digging (fig. 2(c)).

Digging behaviour of F₁ hybrid larvae from crossing the *yellow* strain (non-digger strain) with the Oregon R-C and *taxi* strains (digger strains) is shown in fig. 3 (a, b). After 72 hours of development F₁ hybrid larvae obtained from crossing the *yellow* and Oregon R-C strains dig medium like their digger parents of the Oregon R-C strain (fig. 3 (a)). On the other hand, F₁ hybrid larvae from the cross between *yellow* and *taxi* flies show a burrowing behaviour greater than that of their digger parents of the *taxi* strain, suggesting again the existence of hybrid vigour for digging (fig. 3 (b); see also fig. 2 (c)).

Fig. 4 shows the development of digging behaviour in F₁ hybrid larvae originating from the cross between two digger strains: the Oregon R-C and *taxi*. As seen in fig. 4, F₁ hybrid larvae dig into medium like larvae of the Oregon R-C strain. An inspection of means of stained larvae of each one of the F₁ reciprocal crosses studied in this

Table 1 The larval digging behaviour of the sibling species *D. pavani* and *D. gaucha*. Digging of the medium was measured for different time intervals. χ^2 compares number of stained larvae of these species in each interval of time

	hours of development											
	60	72	84	96	108	120	132	144	156	168	180	192
<i>D. pavani</i>												
Unstained larvae	184	247	385	355	152	79	71	50	9	—	—	—
Stained larvae	—	3	6	95	146	264	355	290	411	396	330	359
% stained larvae	—	0.01	1.80	18.80	48.15	74.78	87.42	86.42	97.53	100.00	100.00	100.00
Variance of stained larvae	—	2.39	3.95	2.22	4.95	6.82	3.22	2.01	0.28	—	—	—
<i>D. gaucha</i>												
Unstained larvae	366	380	377	336	376	292	200	79	76	23	22	25
Stained larvae	—	—	—	—	15	46	102	242	212	292	176	253
% stained larvae	—	—	—	—	5.52	13.20	35.03	75.37	75.12	89.83	89.11	91.00
Variance of stained larvae	—	—	—	—	1.31	4.62	7.60	3.92	9.74	1.74	1.63	1.23
χ^2 test (df = 1)	—	—	—	—	19.26	27.56	18.57	1.03	9.51	—	—	—
	—	—	—	—	p < 0.01	p < 0.01	p < 0.01	p > 0.05	p < 0.01	—	—	—

Figure 2 The development of digging behaviour in hybrid larvae from the cross of the *vestigial* (■) strain with the Oregon R-C (□), *taxi* (●) and *yellow* (○) strains. (a) Oregon R-C male × *vestigial* female (Δ); Oregon R-C female × *vestigial* male (▲). (b) *taxi* male × *vestigial* female (Δ); *taxi* female × *vestigial* male (▲). (c) *yellow* male × *vestigial* female (Δ); *yellow* female × *vestigial* male (▲).

research revealed no substantial differences between them (arcsine transformation). For instance, at 84 hours of development t values ranged from 0.120 to 1.714 (P , two tailed >0.05); at 108 hours of development t fluctuated between 0.029 and 0.443.

(iii) *Locomotor activity in larvae of digger and non-digger strains*

Burrowing behaviour entails considerable larval locomotor activity. Table 2 shows locomotor

behaviour of larvae at 108 hours of development of the Oregon R-C and *vestigial* strains and of larvae of the high and low digging lines (see Godoy-Herrera, 1978). Larvae of the Oregon R-C strain (digger strain) and *vestigial* larvae (non-digger strain) do not show substantial differences in locomotion. However, larvae of the low digging line show a locomotor behaviour lower than that of larvae of the base population (the Oregon R-C strain). Larvae of the high digging line exhibit higher locomotion than larvae of the unselected base population (the Oregon R-C strain).

Figure 3 The development of digging behaviour in hybrid larvae obtained from the cross of the yellow (○) strain with the Oregon R-C (□) and taxi (●) strains. (a) Oregon R-C male × yellow female (Δ); Oregon R-C female × yellow male (▲). (b) taxi male × yellow female (Δ); taxi female × yellow male (▲).

(iv) Puparium forming behaviour of larvae of the high and low digging lines

Fig. 5 indicates that, after the F15 generation of selection for low digging, larvae of this line tend to form puparia away from the surface of medium, while those of the high digging line pupariate near

on the surface of medium, showing similar behaviour to that of larvae of the base population (the Oregon R-C strain).

DISCUSSION

This research shows that larval dispersal patterns of *D. melanogaster* within the substrate and of the sibling species *D. pavani* and *D. gaucha* depend on larval development (fig. 1 and table 1). In general, bigger older larvae seem to go into the culture medium deeper than smaller young larvae. In *D. melanogaster*, larval demands for food and

Figure 4 The development of digging behaviour in hybrid larvae obtained by crossing the Oregon R-C (□) and taxi (●) strains. Oregon R-C male × taxi female (Δ); Oregon R-C female × taxi male (▲).

Table 2 Larval locomotor activity of the digger Oregon R-C strain and the non-digger *vestigial* strain and of the high and low digging lines (F20 generation). These last populations had been genetically selected from the Oregon R-C strain (see Materials and Methods).

Strain	Mean of larval locomotor activity (cm)	Number of larvae tested	"t" test respect to Oregon R-C strain	degrees of freedom
Oregon R-C	47.16 ± 2.46	47	—	—
high digging line	58.20 ± 2.38	50	3.311*	95
low digging line	38.40 ± 2.06	47	2.811*	92
<i>vestigial</i>	42.00 ± 2.17	31	0.466	76

* $p < 0.05$.

Figure 5 Changes in larval pupariation behaviour of the lines genetically selected for low (Δ) and high ($\circ$) larval digging behaviour. Choice pupariation site was measured as a mean distance from the culture medium (see Materials and Methods).

space are also age-related (Sewell *et al.*, 1975; Green *et al.*, 1983; Godoy-Herrera *et al.*, 1984; Sokolowski *et al.*, 1984). Variations in digging shown by larvae of *Drosophila* with similar nutritional requirements could be of importance for understanding coexistence of these species in the wild (Barker, 1971). Atkinson (1979) has argued that competition for food and space between *Drosophila* species which breed at the same sites could be prevented through their larval behaviour which may lead to use of the resources of the environment in different ways.

When observing the number of larvae with and without charcoal, it was frequently noted that nearby or in the same tunnel made in the medium by a larva of *D. melanogaster* there could be found other individuals, suggesting that these preadults burrow in groups. Such larval aggregations were never observed in the case of *D. pavani* and *D. gaucha*. The viability of *D. melanogaster* larvae is not affected by their own biotic residues, but the residues of larvae of *D. pavani* and *D. gaucha* interfere with the development of these species (Budnik and Brncic, 1975; 1976). Individually digging the substrate might be a behavioural adaptation of larvae of *D. pavani* and *D. gaucha* which has evolved to decrease the effects of their own biotic wastes on viability.

Larvae of the Oregon R-C, *taxi*, *yellow* and *vestigial* strains and their hybrids also show digging behaviour related to age, as measured by the

percentage of stained larvae between 60 to 120 hours of development. Digging of the culture medium is very fast between 72 to 96-hours-old, decreasing after 108 hours (figs. 2 to 4). An increase of larval digging behaviour with age could allow for access to food at lower levels of the substrate expanding the space available to larvae, preventing larval inter- and intraspecific competition for these resources. The results also suggest dominance effects with some heterosis for digging (figs. 2(a), 2(b), 3(a) and 4), and that perhaps an epistatic component is present (figs. 2(c) and 3(b)). Characters closely related to fitness show a genetical architecture with allelic and non-allelic interactions and with little or no additive genetic variance (Broadhurst and Jinks, 1974). Godoy-Herrera *et al.* (1984) have shown that locomotor activity of *D. melanogaster* larvae increases with larval age as a result of growth in body size and of changes in the epigenetic relationships between genes controlling this behaviour.

Of the four strains of *D. melanogaster* studied in this paper Oregon R-C larvae showed a greater tendency to dig within the culture medium. This may reflect evolutionary pressures on digging behaviour of larvae of this strain. Such an inference is based on the fact that females of the Oregon R-C strain are very prolific. Bottles in which the Oregon R-C strain is cultured show numbers of larvae and pupae higher than those in which the *taxi*, *yellow* and *vestigial* strains are kept. These

mutations decrease viability (Lindsley and Grell, 1972). The consistency of the culture medium is rapidly altered due to tunnelling by Oregon R-C larvae, suggesting that a large number of these preadults are present. This suggests that larvae of the Oregon R-C strain may be under a continuous pressure to dig caused by high numbers of larvae hatching from eggs deposited by the females. Under continuous directional selection a gene would be expected to become dominant (Fisher, 1930).

Differences in locomotor behaviour may explain why there are differences in the amount of digging of the culture medium by larvae of the high and low digging lines (table 2). However, at 108 hours of age larvae of the digger Oregon R-C strain and those of the non-digger *vestigial* strain show a similar level of locomotion (table 2). I have found that larvae of the *vestigial* strain kept in darkness dig the culture medium substantially more than controls maintained in light (Godoy-Herrera, 1982). Perhaps digging of the culture medium by *D. melanogaster* larvae may also be influenced by their photoresponse. More experiments are necessary to understand the relationships between larval age, locomotion and photoresponse in relation to the behavioural mechanisms of digging.

Selection for low digging behaviour has also modified pupariation behaviour of larvae of this line (fig. 5). Sokal (1966) reported that larvae selected to pupariate away from medium tend to remain in the upper zone of substrate when feeding. These findings suggest that genes controlling these two larval behaviours of *D. melanogaster* could be linked on the same chromosomes. On the other hand, the fact that larvae with a comparatively low locomotor activity (table 2) pupariate away from the medium confirms that other factors such as humidity are important in selection of pupariation sites (Sameoto and Miller, 1968; Schlager, 1960).

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