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Dispersal and prepupation behavior of Chilean sympatric *Drosophila* species that breed in the same site in nature

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We investigated dispersal patterns of *Drosophila* larvae searching for pupation sites over three substrates to determine the role of spatial heterogeneity and presence of other species on prepupation behavior. We used *D. melanogaster*, *D. hydei*, and *D. pavani* whose parents emerged from apples collected in one orchard. Each species showed different preferences for substrates on which to pupate, particularly in the presence of another *Drosophila* species. Larval locomotion rate and turning behavior in *D. melanogaster*, *D. hydei*, and *D. pavani* were modified depending this upon the type of substrate (agar and sand) on which the larvae crawled. These two behaviors are involved in dispersal and aggregation of pupae. Distance between pupae of the same species decreases when larvae of another species pupate on the same substrate. Aggregated distributions over the substrates lead to patches with few or no individuals. These could serve as pupation sites for other *Drosophila* species that, in nature, also emerge from small breeding sites. *Key words:* breeding sites, pupation behavior, sympatric *Drosophila* species. *[Behav Ecol]*

Dispersal is a life history trait that affects the distribution and abundance of species, with consequences for community structure (Dieckermann et al., 1999). Dispersal may also reduce intra- and interspecific competition for food and space, contributing to coexistence of species (Shorrocks and Bingley, 1994). Research on the behavioral basis of dispersal in relation to utilization of food and space could also help to discover which behavioral patterns contribute to this coexistence in the wild (Nunney, 1990) and may reveal how local guilds that share common resources can coexist and persist through time (Martin and Martin, 2001).

In *Drosophila*, larval patterns of movement are central to understanding foraging strategies and selection of pupation sites (Godoy-Herrera and Silva-Cuadra, 1998; Sokolowski, 1986). However, most studies on larval prepupation behavior of *Drosophila* have used food vials as the substrate. Pupation site preference has been measured by the distance between the surface of the substrate and the pupa location (review in Singh and Pandey, 1993). This experimental design provides little insight into features of the environment that may regulate dispersal patterns of larvae searching for pupation sites. Wong et al. (1985), Godoy-Herrera et al. (1989), and Godoy-Herrera and Silva-Cuadra (1997, 1998) observed that larvae of *Drosophila melanogaster*, *Drosophila pavani*, *Drosophila gaucha*, and of the reciprocal interspecific hybrid between the latter two species react to humidity, light, and to substrate texture and consistency. Nevertheless, no studies link prepupation behavior of *Drosophila* with larval dispersal patterns in heterogeneous environments and coexistence of species that breed in the same sites in the wild.

Larval dispersal behavior of *Drosophila* has a genetic component, which is important in the colonization of new niches and the expansion of populations (de Souza et al., 1970). Aspects of choice of pupation site by *Drosophila* also have a genetic basis (Grossfield, 1978; Sokolowski et al., 1986). For example, pupation by *D. melanogaster* on dry substrates outside the food cup is dominant over pupation inside the cup; there is also additive variation (Godoy-Herrera et al., 1989). Singh and Pandey (1993) found that pupation height in shell vials in *Drosophila ananassae* is under polygenic control and most of the variance is additive. The type of pupation site selected by larvae also affects pupal survival of *D. melanogaster* (Rodriguez et al., 1992; Sokal, 1966). When placed on dry substrates, *D. pavani* and *D. gaucha* pupated both outside and inside the food cup, while the interspecific hybrid larvae did so only inside the cup (Godoy-Herrera and Silva-Cuadra, 1997). *Drosophila simulans*, *Drosophila hydei*, and *Drosophila busckii*, which share the same breeding sites in the Central Valley of Chile, show different substrate preferences to form puparia. For example, *D. busckii* larvae select humid substrates with a smooth surface for pupation, whereas *D. simulans* larvae select humid substrates with a rough surface (Godoy-Herrera and Silva-Cuadra, 1998).

In this work we propose that the larval movement patterns of *Drosophila* species observed to form puparia contribute to their coexistence. To test this hypothesis, we compared substrate preferences of larvae from of each three species that had emerged from the same rotten apples collected in one orchard in the presence and absence of another *Drosophila* species. We also recorded movement patterns on two substrates of larvae while searching for pupation sites. Additionally, we measured aggregation of pupae in the presence and absence of another *Drosophila* species. These studies enabled us to explore how habitat heterogeneity interacts with the presence of another *Drosophila* species to influence movement and dispersal of larvae searching for pupation sites.

METHODS

Subjects

We used wild type *D. melanogaster* (subgenus *Sophophora*, *melanogaster* group), *D. hydei* (subgenus *Drosophila*, *repleta* group), and *D. pavani* (subgenus *Drosophila*, *mesophragmatica*

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Table 1

Experiments performed to study *D. melanogaster*, *D. hydei*, and *D. pavani* larval preferences for substrates during pupation

Experiment and rearing cup with	Replicates	Bottom of the boxes outside of food cup	Larval preferences for pupation substrate outside food cup
(1) Medium	4	½ perspex / ½ agar	Dry (perspex) and moist (agar) substrates
(2) Medium	4	½ dry sand / ½ agar	Dry (sand) and moist (agar) substrates
(3) Medium	4	½ dry sand / ½ perspex	Two distinct dry substrates (sand and perspex)

The larvae could choose to pupate inside (on the medium of the rearing cup) or outside (on the perspex [plastic], agar, and sand substrates) of the rearing cup. Populations used were collected in an orchard (Chillán, Chile).

group). Flies were collected in April of 1999 (Autumn in Chile) in Chillán, 420 km south of Santiago. In this season Chilean populations of *Drosophila* reach their peaks of abundance (Brncic, 1980). Collections were made in an orchard of the University of Concepción (Faculty of Agronomy). This is a humid site in which ornamental plants, native vegetation, and tomatoes and grapes grow together with cherry, plum, medlar, apple, and peach trees. Once these fruits fall on the ground, *Drosophila* use them as breeding sites (Brncic, 1980, 1987).

We used eggs and larvae of *D. melanogaster*, *D. hydei*, and *D. pavani* whose parents had emerged from 10 overripe apples (Red Delicious variety) collected in an orchard. Ten of these fruits were individually deposited into 500 cc flasks kept at 22°C in the laboratory. The stock population for each of the three species was established from a mixture of 10 males and 10 females emerged from each of the 10 fruits (i.e., a total of 200 individuals). The amount of genetic variability in the stocks was not estimated. Other *Drosophila* species (*D. simulans*, *D. repleta*, *D. immigrans*, *D. busckii*, *and D. subobscura*) also emerged from the collected apples. *D. melanogaster*, *D. hydei*, and *D. pavani* pupae can be distinguished morphologically. The *D. melanogaster* pupae are brown-yellow in color and measure 3.50 ± 0.05 mm ($N = 120$), whereas those of *D. hydei* and *D. pavani* measure 4.50 ± 0.01 mm ($N = 120$) and are brown-black in color. *D. hydei* and *D. pavani* pupae can be distinguished by their horn shapes; *D. hydei* pupae have curved horns while *D. pavani* pupae have V-shaped horns. Pupae of *D. simulans*, *D. repleta*, *D. immigrans*, *D. busckii*, and *D. subobscura*, which also emerged from the apples, could not be distinguished morphologically from those of the species used in the present experiments. No more than four generations had elapsed between the establishment of the populations and their use in the present study. The stocks were kept in half-pint bottles containing 50 cc of Burdick's medium (1954) at 24°C.

Eggs and larvae collection

Groups of 60–70 inseminated females were allowed to lay eggs for 3–4 h on plastic spoons filled with culture Burdick's medium. Eggs were collected with a dissecting needle. For the single species experiments, 100 eggs were sown on individual 2.0 × 2.0 cm cups filled with Burdick's medium. For the experiments using two species, 50 24-h old larvae of each of the species of the following dyads were placed in the same cup: (1) *D. melanogaster* and *D. hydei*, (2) *D. melangaster* and *D. pavani*, and (3) *D. hydei* and *D. pavani*. Previous observations had shown that mortality of *D. hydei* and *D. pavani* was over 95% when their eggs were sown together with those of *D. melanogaster* in the same rearing cup. However, mortality decreased to 25–30% when 24-h old larvae of *D. melanogaster* were deposited together with 24-h old larvae of either of the other two species. This is comparable to that obtained in single species cultures. *D. melanogaster* egg chorion may contain some substances that increase mortality of *D. pavani* and *D. hydei* preadults.

Behavioral experiments

The experimental design was modified from de Souza et al. (1970) (Table 1). We used 10 × 10 × 10 (wide × long × high) cm transparent plastic boxes as described by Godoy-Herrera et al. (1989). All the boxes were situated in the same area of the rearing chamber to avoid variations in illumination and temperature.

Pupation substrate preferences

Pupation substrate preferences of *D. melanogaster*, *D. hydei*, and *D. pavani* were tested by offering the larvae three different substrate combinations with two treatments in each. The treatments were each species alone versus presence of another *Drosophila* species, then the three possible pairwise substrate combinations (Table 1). Each treatment of each substrate combination contained four replicates. For each of the species, a set of eight plastic boxes were filled with 3% agar to a depth of 2 cm. Then, an area of agar measuring 10.0 × 5.0 cm was removed along one side to leave a dry surface. In the first experiment, a 2.0 × 2.0 cm food cup was placed at the bottom of four boxes. Thus, larvae could choose to pupate either on agar, plastic, or in the rearing cup (experiment 1, Table 1). In the second experiment, the plastic part of four boxes was filled with yellow dry sand forming a 2-cm thick layer (experiment 2, Table 1). The number of pupae outside (plastic, agar, and sand) and inside of the rearing cup was recorded prior to eclosion. Finally, larval preferences for two kinds of dry substrates were tested (experiment 3, Table 1). Floor measuring 10.0 × 5.0 cm of four plastic boxes was filled to a depth of 2 cm with dry yellow sand. To keep the sand to one side of the box, two pieces of the same type of plastic measuring 4.0 × 2.0 cm (length × height) were placed between the cup and each of the opposite sides of the box. The boxes were maintained for six days (*D. melanogaster*) and 12 days (*D. hydei* and *D. pavani*) at 24°C. These substrates allowed control of contamination by fungi and bacteria.

Larval dispersal distances

Larval dispersal activity of the three species on each of the substrates was evaluated in the presence and absence of another *Drosophila* species. The distance between the pupae found on the surface of the plastic, agar, and sand and the center of the cup (experiments 1, 2, and 3, Table 1) was recorded. Normality of the frequency distributions was also tested under the presumption that a departure from normality might indicate a tendency for conspecific larvae to pupate near each other.

Nearest neighbor analysis

Pupal aggregation in the substrates of each of the three species was recorded, in the presence and absence of another

species. First, the position of each of the pupae on the substrates outside of the rearing cup was registered. Then, the distance to the nearest neighbor was measured using a 0.5 cm Cartesian grid. In the experiments with two species, we also recorded species identity of the nearest neighbor pupae. Pupae distributions were analyzed using the nearest neighbor method of Clark and Evans (1954). The average distance to the nearest neighbor of the same species (rA) obtained in the single species treatments was compared with the expected value (rE) for the same number of individuals randomly distributed on an area of equal size ($rE = \frac{1}{2}\sqrt{\rho}$), where ρ is the pupal density. The ratio $R = (rA/rE)$, reflects the form of the spatial distribution of individuals (aggregated, random, overdispersed). Its value ranges between $R = 0$ (maximum aggregation) and $R = 2.15$ (uniform). When individuals are randomly distributed then $R = 1$. The analysis was repeated for the mixed species treatments. Then, we compared *R*-values from single and mixed species treatments using ANOVA (Clark and Evans, 1954).

Searching patterns

Pupation site choice by *Drosophila* larvae depends on the larval patterns of movement on different substrates (Sokolowski et al., 1986). Samples of 50 late third instar larvae of *D. melanogaster*, *D. hydei*, and *D. pavani* were collected from the walls of the culture bottles. Larvae were individually deposited on agar or dry sand. Each larva was tested on new agar and sand. Larvae might remain motionless on the substrates. Once each larva began to move, the trail made by each larva was drawn for a period of two min using a Wild M5 camera lucida. The trail length was measured as described in Sokolowski (1980). These measurements provided an estimate of locomotion. Larval turning behavior was also estimated by counting the number of directional changes in each trail.

Statistical analysis

We used a G-test of Independence (Sokal and Rohlf, 1995) to determine whether the number of pupae inside the rearing cup in each treatment and substrate combination (Table 1) was significantly different from that found outside the cup. We examined homogeneity for replicates within substrate combinations (single and mixed species) using the R × C test of Independence (Sokal and Rohlf, 1995).

Normality of pupae distributions outside the rearing cup (plastic, agar, and dry sand) was examined using a Kolmogorov-Smirnov test, and homogeneity of variances was also determined with Bartlett's test in the single and mixed species treatments. The skewness (g1) and kurtosis (g2) of the data were also calculated. These statistics were used to estimate aggregation.

RESULTS

The replicates in each treatment and substrate combinations were not shown to be significantly different. So, the data were pooled as shown below. However, the data were not normally distributed (Kolmogorov-Smirnov test, $D = 86$, $p < .05$), although variances were homogeneous (Bartlett's test, $\chi^2_{0.05} = 7.82$, df = 3, $p > .05$). Therefore, nonparametric statistics were used to analyze the data.

Larval substrate preferences

In the single species experiments, *D. melanogaster* larvae pupated on dry sand whereas *D. hydei* and *D. pavani* larvae pupated on agar (Figure 1, first row). The distributional

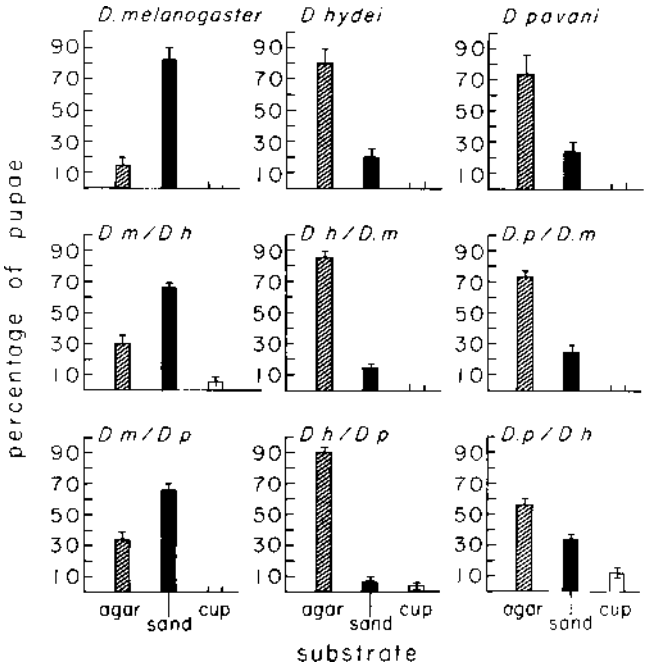

Figure 1
Substrate preferences (agar, dry sand, and cup) ($X \pm SE$) to pupate of *D. melanogaster*, *D. hydei*, and *D. pavani* larvae in the absence (first row) and presence (second and third rows) of another *Drosophila* species, pooled per replicates. Each experiment was replicated four times. Preference for a substrate was calculated as the percentage of all pupae found on each substrate used. Dm/Dh: *D. melanogaster* in the presence of *D. hydei*; Dm/Dp: *D. melanogaster* in the presence of *D. pavani*; Dh/Dm: *D. hydei* in the presence of *D. melanogaster*; Dh/Dp: *D. hydei* in the presence of *D. pavani*; Dp/Dm: *D. pavani* in the presence of *D. melanogaster*; Dp/Dh: *D. pavani* in the presence of *D. hydei*. Total number of pupae in each experiment fluctuated between 280 and 300.

pattern of *D. melanogaster* pupae was not modified by the presence of *D. hydei* or *D. pavani* preadults (Figure 1, first column). On dry sand, *D. hydei* and *D. pavani* pupae distributions also did not differ in the absence and presence of another *Drosophila* species (Figure 1; G-test of Independence values were all below critical value, $\chi^2_{0.05} = 5.99$, df = 2, NS).

When the rearing cup was surrounded by agar and plastic (Figure 2), most *D. melanogaster*, *D. hydei*, and *D. pavani* pupae were observed on agar. In the presence of *D. hydei* and *D. pavani*, *D. melanogaster* larvae also used the plastic to pupate (G-test of Independence, presence versus absence of *D. hydei* and *D. pavani*, $\chi^2 = 20.83$ and 22.18, respectively, df = 2, $p < .01$). In contrast, most *D. hydei* and *D. pavani* larvae left the cup in the presence of the other *Drosophila* species pupating (over 70 %) on agar (Figure 2). Nevertheless, in the presence of *D. melanogaster* larvae, about 11% of *D. hydei* pupae were detected on the plastic (G-test of Independence, *D. hydei*, presence versus absence of *D. melanogaster*, $\chi^2 = 10.21$, df = 2, $p < .05$). About 30% of *D. pavani* pupae were also found on the plastic when *D. hydei* was present (Figure 2; G-test of Independence, *D. pavani*, presence versus absence of *D. hydei*, $\chi^2 = 15.22$, df = 2, $p < .05$).

When the cup was surrounded by dry sand and plastic (experiment 3; Table 1), most *D. melanogaster* and *D. hydei* larvae pupated in the rearing cup (Figure 3, first row). In contrast, 100% of *D. pavani* pupae were detected outside the cup on the sand (Figure 3, first row). This situation changed in the presence of another *Drosophila* species. For example, when *D. hydei* or *D. pavani* were present, most of

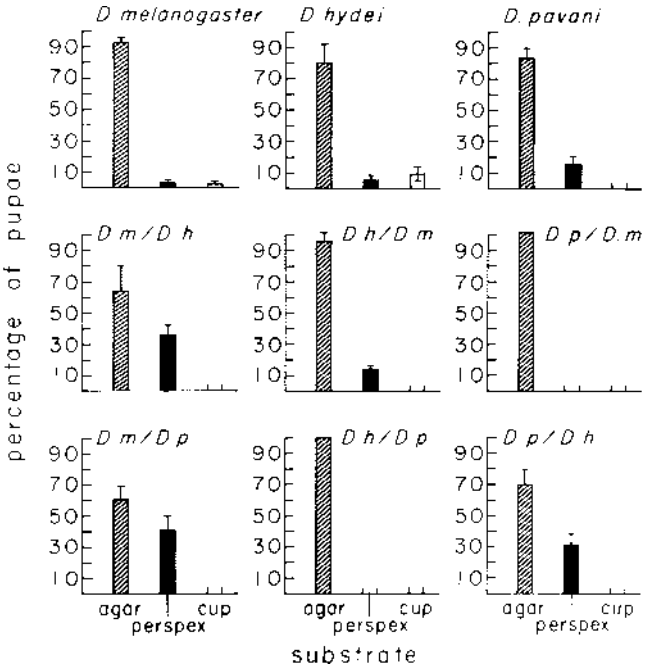

Figure 2
Substrate preferences (agar, perspex [plastic], and cup) ($X \pm$ SE) to pupate of *D. melanogaster*, *D. hydei*, and *D. pavani* larvae in the absence (first row) and presence (second and third rows) of another *Drosophila* species. Other details as in Figure 1.

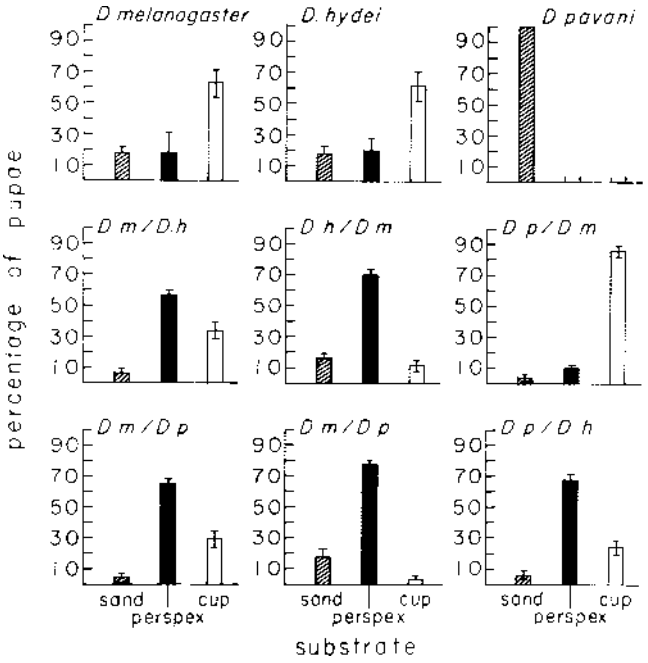

Figure 3
Substrate preferences (sand, perspex [plastic] and cup) ($X \pm$ SE) to pupate of *D. melanogaster*, *D. hydei*, and *D. pavani* larvae in the absence (first row) and presence (second and third rows) of another *Drosophila* species. Other details as in Figure 1.

D. melanogaster pupae were on the plastic (Figure 3, first column; G-test of Independence: (1) presence versus absence of *D. hydei*, $\chi^2 = 24.36$, df = 2, $p < .001$; (2) presence versus absence of *D. pavani*, $\chi^2 = 32.18$, df = 2, $p < .01$).

In the case of *D. hydei*, most of the pupae were detected outside the cup on the plastic when *D. melanogaster* or *D. pavani* larvae were present (Figure 3, second column; G-test of independence: (1) presence versus absence of *D. melanogaster*, $\chi^2 = 31.13$, df = 2, $p < .01$; (2) presence versus absence of *D. pavani*, $\chi^2 = 29.47$, df = 2, $p < .01$).

D. pavani larvae tended to pupate in the rearing cup when *D. melanogaster* was present (Figure 3, third column). In the presence of *D. hydei*, *D. pavani* larvae pupated on the plastic (G-test of Independence values exceeded the critical value, $\chi^2 = 5.99$, df = 2, $p < .01$; Figure 3).

Aggregation in the presence and absence of another *Drosophila* species

Outside the rearing cup (the plastic, agar, and dry sand) larvae of *D. melanogaster*, *D. hydei*, and *D. pavani* formed puparia near other larvae, as shown by the distributional pattern of pupae on the substrates (skewness [g1] and kurtosis [g2] values fluctuated between 10.54 and 32.14). The *Z*-values calculated to evaluate the goodness-of-fit of pupae distributions with respect to a normal distribution exceeded the critical two-tailed value (all $Z > Z_{0.05} = 1.96$, $p < .05$).

Larval aggregation behavior of *D. melanogaster*, *D. hydei*, and *D. pavani* on the plastic, agar, and sand was also estimated by recording distances (cm) between pupae of one species in absence or presence of *Drosophila* species (Table 2). The distance between the nearest neighbor pupae of the same species decreased in the presence of another *Drosophila* species (Kruskal-Wallis one way ANOVA values were all greater than the critical value H = 10.88, df = 2, $p < .001$). Nearest neighbor analysis also yielded significant values of *R* (Clark and Evans, 1954), indicating that the pupae are aggregated ($R < 1$; Kruskal-Wallis test values exceeded the critical value H = 5.99, df = 2, $p < .05$). In the presence of another *Drosophila* species, the calculated *R*-values were statistically lowest than the *R*-values yielded in the single experiments (Kruskal-Wallis one way ANOVA values were greater than the critical value H = 5.99, df = 2, $p < .05$).

Larval dispersal and patterns of movement

In the absence of another *Drosopohila* species, the distance at which larvae of *D. melanogaster*, *D. hydei*, and *D. pavani* pupated varied depending on the substrate (Table 3). When another *Drosophila* species was present, most larvae of *D. melanogaster*, *D. hydei*, and *D. pavani* modified their dispersal activities. The magnitude of change in dispersal depended on the type of substrate and which of the species was present (Table 3). For instance, when alone and on agar *D. melanogaster* larvae tended to pupate at 4.45 ± 0.32 cm away from the rearing cup, whereas in the presence of *D. pavani* they pupated at a distance of 1.36 ± 0.82 cm (Table 3). In contrast, on the same substrate, in the absence of *D. melanogaster* most *D. pavani* pupae were detected at 5.63 ± 1.15 cm away from the cup, and they were detected at 5.86 ± 1.15 cm when *D. melanogaster* was present (Table 3). For most of the comparisons of presence versus absence of another species, the Kruskal-Wallis test values exceeded the critical value (H = 3.84, df = 1, $p < .05$). We conclude, therefore, that *Drosophila* larvae that coexist with other species in the same breeding sites respond to substrate features and to the presence of larvae of other species of the genus, modifying their dispersal patterns.

To understand better how *D. melanogaster*, *D. hydei*, and *D. pavani* third instar larvae disperse over the agar and sand, we drew the trails made by the preadults on those substrates. The length of the trails was an estimation of locomotion, and mean turning behavior was calculated from number of

Table 2

Mean distance ($X \pm$ SE) to nearest neighbor pupa of the same species

Species	Distance from a pupa to the nearest neighbor pupa of the same species		
	Experiment 1	Experiment 2	Experiment 3
	½ agar / ½ perspex	½ dry sand / ½ agar	½ dry sand / ½ perspex
D. melanogaster			
(1) alone (control)	0.38 ± 0.07	0.50 ± 0.36	0.34 ± 0.06
(2) in the presence of *D. hydei*	0.09 ± 0.04	0.20 ± 0.11	0.16 ± 0.04
(3) in the presence of *D. pavani*	0.12 ± 0.08	0.24 ± 0.13	0.16 ± 0.04
D. hydei			
(1) alone (control)	0.48 ± 0.05	0.28 ± 0.04	0.30 ± 0.02
(2) in the presence of *D. melanogaster*	0.10 ± 0.03	0.12 ± 0.03	0.10 ± 0.03
(3) in the presence of *D. pavani*	0.13 ± 0.09	0.15 ± 0.03	0.11 ± 0.01
D. pavani			
(1) alone (control)	0.29 ± 0.03	0.40 ± 0.04	— (100% pupae in the rearing cup)
(2) in the presence of *D. melanogaster*	0.36 ± 0.48	0.34 ± 0.06	0.07 ± 0.02
(3) in the presence of *D. hydei*	0.23 ± 0.03	0.17 ± 0.06	0.33 ± 0.13

Individuals were of *D. melanogaster*, *D. hydei*, and *D. pavani*. The populations were collected in an orchard (Chillán, Chile). On perspex (plastic) and agar pupae are found on the surface; larvae dig into the sand, pupating underneath. Distances are expressed in cm.

changes in direction shown by the trails. On agar, *D. hydei* and *D. pavani* larvae exhibited similar rates of locomotion (Kruskal-Wallis test value H = 1.68, df = 1, NS); *D. melanogaster* larvae move slowly on this substrate (Kruskal-Wallis test values: (1) *D. melanogaster* versus *D. hydei*: H = 12.16, df = 1, $p < .01$); (2) *D. melanogaster* versus *D. pavani*: H = 13.11, df = 1, $p < .01$). On agar, turning rates of *D. melanogaster* and *D. pavani* were lower than that of *D. hydei* (Kruskal-Wallis test values: (1) *D. melanogaster* versus *D. pavani*: H = 2.63, df = 1, NS; (2) *D. melanogaster* versus *D. hydei*: H = 16.61, df = 1, $p < .01$; (3) *D. hydei* versus *D. pavani*: H = 8.37, df = 1, $p < .01$).

On the sand, *D. melanogaster* and *D. pavani* larvae decreased their locomotion, whereas *D. hydei* preadults showed an increase in this behavior (Table 4; Kruskal-Wallis test values: (1) *D. melanogaster* versus *D. pavani*: H = 1.32, df = 1, NS; (2) *D. hydei* versus *D. melanogaster*: H = 34.28, df = 1, $p < .01$; (3) *D. hydei* versus *D. pavani*: H = 23.91, df = 1, $p < .01$). All three species show less larval turning behavior on dry sand than on agar (Table 4; Kruskal-Wallis test values were nonsignificant).

DISCUSSION

Cosmopolitan *D. melanogaster* and *D. hydei* and endemic *D. pavani* species bred on the same orchard differ in their larval prepupation behavior. First, the larvae show different preferences for pupation substrates. Second, pupa aggregation increases in the presence of another *Drosophila* species. As a result of these behaviors, pupae of one species are separated from pupae of another species. In addition, late third instar larvae of *D. melanogaster*, *D. hydei*, and *D. pavani* collected in the same orchard pupated at differing depths in the substrate (Godoy-Herrera and Silva-Cuadra, 1997, 1998).

At 24°C, *D. melanogaster* has a shorter larval period (4 days) than that of *D. hydei* and *D. pavani* (7 days). This difference may mean that *D. melanogaster* larvae leaving the rearing cup do not encounter pupae of the other species. Why, then, did *D. melanogaster* larvae modify their pupation behavior when larvae of another species were present? Perhaps recognition of conspecific and alien larvae occurs when first and second instar larvae are feeding. The recognition could be expressed later through pupal aggregation. Further studies are planned to investigate this idea.

In the Central Valley of Chile, a substantial number of *Drosophila* species use decaying apples, plums, and grapes as breeding sites (Brncic, 1987). These abundant, discrete small breeding sites favor interactions among larvae of the same or different species. Environmental heterogeneity may favor species coexistence if *D. melanogaster*, *D. hydei*, and *D. pavani* larvae show different preferences for pupation substrates. Pupa aggregations of one species via recognition of conspecific and alien larvae also reduce competition for

Table 3

Mean distance ($X \pm$ SE) (cm) from the rearing cup at which *D. melanogaster*, *D. hydei*, and *D. pavani* larvae pupate on different substrates in the presence and absence (control) of another *Drosophila* species

Species	Substrate		
	Perspex	Agar	Sand
D. melanogaster			
(1) alone (control)	1.45 ± 0.13	4.45 ± 0.32	0.39 ± 0.02
(2) in the presence of *D. hydei*	2.45 ± 0.26	3.46 ± 0.18	0.37 ± 0.07
(3) in the presence of *D. pavani*	4.58 ± 0.79	1.36 ± 0.82	0.50 ± 0.04
D. hydei			
(1) alone (control)	2.51 ± 0.51	2.50 ± 0.47	4.68 ± 0.07
(2) in the presence of *D. melanogaster*	4.71 ± 0.24	5.79 ± 0.19	2.59 ± 0.23
(3) in presence of *D. pavani*	4.73 ± 0.37	6.63 ± 0.41	3.58 ± 0.71
D. pavani			
(1) alone (control)	—	5.63 ± 1.15	2.79 ± 0.87
(2) in the presence of *D. melanogaster*	6.72 ± 1.07	5.86 ± 0.97	3.89 ± 0.63
(3) in the presence of *D. hydei*	0.45 ± 0.05	3.78 ± 0.94	0.42 ± 0.1

Table 4

Locomotion rate (cm) and N^0 of turns on agar and sand ($X \pm SE$) of *D. melanogaster*, *D. hydei*, and *D. pavani* third instar larvae searching for pupation sites

	Substrate			
	Agar		Sand	
Species	Locomotion rate (cm)	N^0 of turns	Locomotion rate (cm)	N^0 of turns
D. melanogaster	7.57 ± 0.67	5.5 ± 0.72	0.82 ± 0.23	0.20 ± 0.01
D. hydei	11.82 ± 1.32	12.06 ± 1.44	15.78 ± 1.85	0.87 ± 0.27
D. pavani	11.98 ± 1.27	7.67 ± 0.94	1.03 ± 0.32	0.77 ± 0.24

Larval activity was observed until the larvae dug into the substrate over a maximun of 2 min. The strains used were collected in an orchard (Chillán, Chile) (see Materials and Methods and Table 1). Number of larvae = 30.

space. Thus, these two features of *Drosophila* species larval behavior may contribute to their coexistence.

In the wild, ecological conditions of *Drosophila* breeding sites change in a relatively short time (Atkinsons and Shorrocks, 1984; Shorrocks and Bingley, 1994; Shorrocks and Rosewell, 1987). In changing environments organisms may evolve diverse traits characterized by a high phenotypic plasticity (Levins, 1968). Our results show that larval prepupation behavior of *D. melanogaster*, *D. hydei*, and *D. pavani* exhibit such plasticity, as expected given the changing features of decaying fruits used as breeding sites.

On the other hand, genetic polymorphisms in a variable environment are possible when the populations are regulated by density dependence in each habitat (Levene, 1953). Thus, European larvae of *D. melanogaster* that remain in the upper layer of the substrate are more infested by a parasitic *Hymenoptera* than are those that dig into the medium. "Rover" larvae go deeper into the substrate than "sitter" preadults (Carton and David, 1985). In this way, the parasite helps maintain the frequencies of "rover" and "sitter" larvae of *D. melanogaster* in the wild (Carton and Sokolowski, 1992). In contrast, in Chile, monthly collections of *D. melanogaster*, *D. hydei*, *D. pavani*, and other *Drosophila* species for over 40 years have been unsuccessful in revealing any parasitic *Hymenoptera* (Brncic, 1980). Chilean *Drosophila* communities change through the year. This suggests that seasonal fluctuations in physical environmental factors, abundance of breeding sites, and presence of other *Drosophila* species could be important evolutionary pressures to regulate larval dispersal, preferences for pupation substrates and pupa aggregations. Recent field observations on the behavior of third instar larvae that have left natural decaying fruit-breeding *Drosophila* show that highly aggregated pupae distributions are commonly observed in the wild (unpublished data).

Dispersal and aggregation of pupae depended on larval movement patterns. Heterogeneity of the substrates can greatly modify locomotion rate and turning behavior of *D. melanogaster*, *D. hydei*, and *D. pavani* larvae (Table 4). In particular, on the sand, larval movement of *D. melanogaster* and *D. pavani* larvae tends to be slower than on other substrates. However, *D. hydei* larvae increased their locomotion on this substrate. The energetic cost of larval crawling is higher than walking, flying, or swimming (Berringam and Lighton, 1993). As larval dispersal takes place, the forces generated by surface tension over a relatively large area must be overcome, and friction is produced in contact with the surface (Casey, 1991). The frequencies of locomotion, turning, and bending behavior exhibited by *Drosophila* larvae (Green et al., 1983) imply a significant energetic cost. This cost should be lower when the larvae crawl on a moist surface such as agar, and it should be higher on substrates such as sand. We noted that in the case of *D. melanogaster* and *D. pavani* larvae, their movements seemed to decrease as the number of obstacles in the landscape increased. This might be expressed in an increase in larval settlement rates, and thus in pupa aggregation. In contrast, *D. hydei* larvae, which increase their movement on the sand, might pupate and aggregate far from *D. melanogaster* and *D. hydei* pupae.

Spatial heterogeneity influences habitat selection, foraging ecology and space utilization, and refuge from predation (Bond et al., 2000). On the other hand, the interactions between biotic and abiotic influences on habitat use have important implications for coexistence of scramble type competitors species that share common resources (Martin and Martin, 2001). This is the case for *Drosophila* species that appear to occupy similar ecological niches (Powell, 1997). In mixed species tests, pupae of *D. melanogaster*, *D. hydei*, and *D. pavani* showed aggregated distributions over the substrates, creating patches with few or no individuals. These could serve as potential pupation sites for other *Drosophila* species, such as those that also lived in the orchard (*D. simulans*, *D. repleta*, *D. immigrans*, *D. busckii*, and *D. subobscura*).

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