Genetic Analysis of *Drosophila sechellia* Specialization: Oviposition Behavior Toward the Major Aliphatic Acids of Its Host Plant

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Oviposition behavior of the four species in the *Drosophila melanogaster* complex (*D. melanogaster, D. simulans, D. mauritiana, D. sechellia*) was investigated versus natural morinda fruit (the normal resource of *D. sechellia*) and the two major aliphatic acids of this fruit (hexanoic acid, C6, and octanoic acid, C8). Two different experimental techniques were compared. When control and experimental food were set on the same egg laying plate, three species (*D. sechellia, D. mauritiana, D. melanogaster*) exhibited a significant preference for morinda; with aliphatic acids, only *D. sechellia* manifested a preference. With separate oviposition sites, a preference was found in *D. sechellia* for morinda and acids, and a general avoidance behavior in the three other species. Genetic analysis of the behavioral response toward C6 and C8 was done with the two plates technique on *D. sechellia, D. simulans*, F1 hybrids and backcrosses. Significant behavioral differences were observed with major effects due to genotype, concentration and their interaction. Hybrid behaviors were intermediate between those of their parents. In several cases, a qualitative reversal from preference to avoidance was observed with increasing concentration. In F1 flies, a dominance reversal was observed with increasing C8 concentration. Different reaction thresholds in different receptors might explain such observations.

KEY WORDS: Hexanoic acid; octanoic acid; *Morinda citrifolia*; *D. simulans*; dominance reversal.

INTRODUCTION

Numerous phytophagous insects exhibit a more or less strict specialization on their host plant, and the physiological and behavioral mechanisms of those relationships have been extensively investigated (Price, 1984; Harborne, 1989; Thompson, 1994). In the majority of investigated cases, the specialization was only described by comparing different species. Genetic analyses were not possible because

species could not be crossed. Genetic analyses can be performed only when host-races or closely related species can be crossed and produce fertile offspring. Among a few genetic investigations which were carried out between host-plant races or closely related species, we can mention studies on *Rhagoletis* (Feder *et al.*, 1990 a, b), *Papilio* (Thompson *et al.*, 1990) and *Drosophila* (R'Kha *et al.*, 1991, 1997)

The *Drosophila melanogaster* subgroup comprises eight species of Afrotropical origin (Lemeunier *et al.*, 1986; Lachaise *et al.*, 1988) among which two are specialized on a single ressource: *D. erecta* on *Pandanus* and *D. sechellia* on *Morinda*

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citrifolia. D. sechellia, restricted to the Seychelles archipelago (Tsacas and Bächli, 1981), is especially interesting since it hybridizes with 3 related species, namely, *D. melanogaster, D. simulans* and *D. mauritiana*. Moreover, F1 hybrid females with the last two species are fertile (Lachaise *et al.*, 1986) and can be backcrossed to the parental species allowing some genetic analysis (Coyne *et al.*, 1991). Since the 3 related species are generalist (Lemeunier *et al.*, 1986; David *et al.*, 1989), it is reasonable to infer that the specialization of *D. sechellia* occurred progressively in the Seychelles, about half a million years ago (Lachaise *et al.*, 1988). In nature, *D. sechellia* is strictly specialized on the ripe *Morinda citrifolia* fruit (hereafter designated as morinda), although it can be reared on usual Drosophila medium (Louis and David, 1986).

Previous investigations with natural morinda have demonstrated that a major adaptation was the tolerance of *D. sechellia* to the toxicity of morinda (R'Kha *et al.*, 1991; Legal *et al.*, 1992). Several other traits have also been modified in an adaptive way, including a stimulation of oogenesis by morinda, an attraction of adults and an oviposition preference (R'Kha *et al.*, 1991, 1997).

About 50 different chemicals have been identified in the ripe morinda fruit (Farine *et al.*, 1996) among which 2 fatty acids, hexanoic and octanoic acid, represented respectively 19% and 58% of all volatile products. These two acids were shown to be responsible for the toxicity of morinda to most insect species, with so far the unique exception of *D. sechellia* (Legal *et al.*, 1994; Amlou *et al.*, 1997).

The amount of these toxic fatty acids is highly variable among different morinda fruits (Legal *et al.*, 1994). Replacing a complex, natural resource by one or a few well defined chemicals would aid genetic physiological or behavioral investigations. In the present work we analyzed the oviposition behavior of *D. sechellia, D. simulans* and interspecific hybrids with respect to several concentrations of the two fatty acids. Both acids elicited behavioral responses analogous to those obtained with morinda. Significant differences were however observed according to the acid and, more interestingly, qualitative changes from preference to avoidance could also be obtained according to the concentration. Moreover, an abnormal behavior, the preference of the sensitive *D. mauritiana* for morinda (Moreteau *et al.*, 1994), has been shown

to depend on the experimental technique used, but is not elicited by the acids.

MATERIALS AND METHODS

Species and Interspecific Hybrids

Most investigations were done on *D. sechellia* and *D. simulans*. Both strains originated from the Seychelles archipelago and were collected in 1985. Each stock was founded by at least 30 inseminated females, then kept as a mass culture. For some comparisons, we used a *D. melanogaster* strain, also collected in the Seychelles in 1985, and a *D. mauritiana* strain collected on Mauritius island in 1988; both were also founded by more than 30 isofemale lines.

Interspecific hybrids were produced between *D. sechellia* males and *D. simulans* females, since this cross is much easier than the reciprocal one (Lachaise *et al.*, 1986; Lee and Watanabe, 1987; R'Kha *et al.*, 1991). Hybrid males are sterile but the females are fully fertile. These F1 females were crossed to males of each parental species, producing backcross progeny, designated as BCsim (with *D. simulans* males) and BCsech (with *D. sechellia* males).

Parental stocks of the 4 species were reared in 250 ml bottles on a cornmeal-sugar medium seeded with live yeast, at medium temperature (20 ± 2°C). Experimental flies were grown at 25°C, at low larval density, on a high-nutrient, killed yeast food (David and Clavel, 1965). This produces standard-size flies in good physiological condition.

Oviposition Behavior

We used two experimental procedures for investigating oviposition choice in the four species. Ten pairs of adult flies (20 pairs for *D. sechellia* owing to its low fecundity) were isolated a few hours after emergence, and kept in axenic food vials for 3 days at 25°C. They were then transferred in large plastic cages (380 ml) with two movable oviposition plates at the bottom (Fig. 1A). One oviposition plate was filled with normal, control medium, and the other one with an acid supplemented food. Both plates were seeded with live yeast, renewed every day, and eggs counted on each of them. A typical experiment lasted 4 consecutive days. As food we used an Instant Drosophila blue
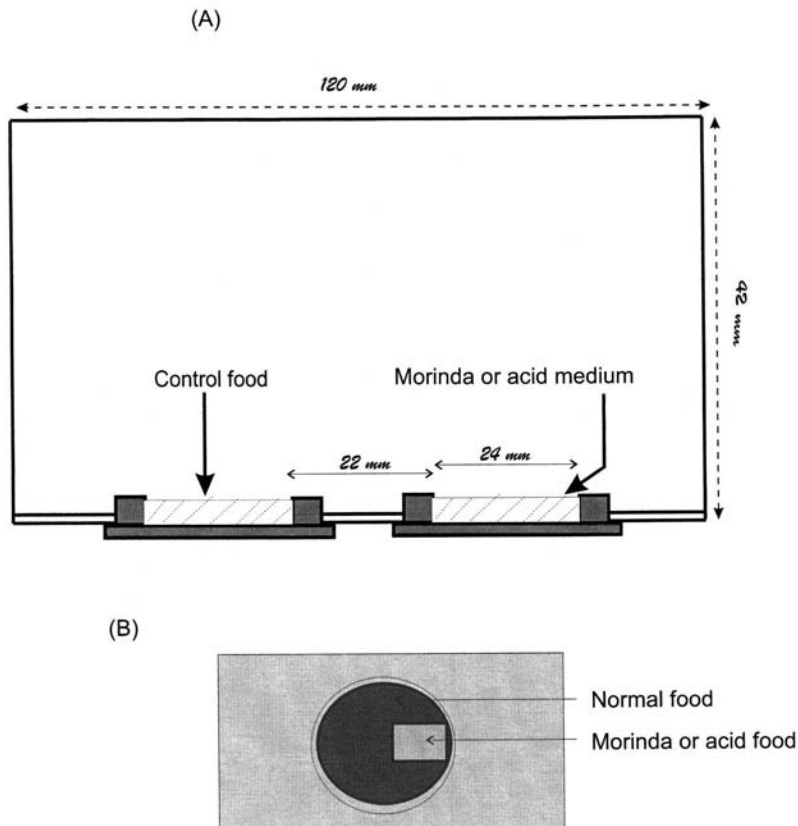
(A)

(B)

Fig. 1. (A) Scheme of an experimental cage (380 ml) with two egg laying plates at the bottom. The two plates are filled with different foods. Minimum distance between the two plates is 22 mm. (B) Scheme of the single plate technique, one compartment (80% of surface) is filled with normal food, the smaller compartment with experimental food. The two compartments are separated by a stripe of paper.

medium (Carolina Biological Supply Company). Four grams of dry medium were thoroughly mixed with 10 ml of cold water. This medium was then distributed in the control egg-laying plates (3.5 g of fresh medium for each plate). New batches of food were prepared daily. A similar procedure was used to produce the acid containing medium. For each acid we used three concentrations with 0.1, 0.3 and 0.5% of acid (weight of acid in percent of fresh weight of medium). The given amount of acid was added with a micropipette, and the batch of food was thoroughly mixed for several minutes, in order to obtain an homogeneous distribution of the acid. This procedure is very important since hexanoic and octanoic acids are poorly soluble in water. The acid food was then transferred to egg-laying plates, and flies were offered a choice between normal and experimental food.

In some experiments the acidic plate was replaced by one filled with natural morinda, which was kept frozen before use. These experiments lasted only one day because *D. simulans* and *D. mauritiana* were very sensitive to morinda toxicity, so that many adults died before the fourth day.

In previous studies (R'Kha *et al.*, 1991; Moreteau *et al.*, 1994) oviposition behavior versus natural morinda was studied in a small cage (92 ml) with a single egg-laying plate. The plate was filled with a corn meal-sugar food. Then 20% of the surface was covered with fresh morinda fruit, and eggs were counted separately on morinda and on control food. We used a similar procedure with the Instant Drosophila medium instead of corn meal-sugar food. This instant food, prepared with cold water, is quite soft and it was impossible to conveniently cover a given area with either morinda or acidic

Table I. Oviposition Behavior of the 4 Sibling Species Versus Ripe Morinda Fruit or Laboratory Food with 0.5% of Acid: Influence of the Experimental Technique (see Fig. 1)[a]

Species	Food	Two oviposition plates			Single oviposition plate		
		Mean % eggs (±SE)	N	Preference index (±SE)	Mean % eggs (±SE)	N	Preference index (±SE)
D. sechellia	Morinda	98.43 ± 0.63	2390	1.97 ± 0.01	91.60 ± 1.38	1077	4.58 ± 0.07
	C6	94.46 ± 2.37	972	1.87 ± 0.05	76.49 ± 3.40	2234	3.59 ± 0.17
	C8	62.99 ± 7.13	1255	1.26 ± 0.14	49.32 ± 3.91	658	2.47 ± 0.20
D. simulans	Morinda	0	2951	0	0.62 ± 0.31	5853	0.03 ± 0.02
	C6	0	3639	0	0	5779	0
	C8	0	3896	0	3.21 ± 1.01	6320	0.16 ± 0.06
D. mauritiana	Morinda	0	2891	0	40.31 ± 1.59	3182	2.02 ± 0.08
	C6	20.45 ± 4.36	3833	0.41 ± 0.09	13.22 ± 1.90	3318	0.66 ± 0.09
	C8	4.47 ± 0.75	4148	0.09 ± 0.01	16.73 ± 1.45	2610	0.84 ± 0.07
D. melanogaster	Morinda	0	6256	0	40.42 ± 4.45	3055	2.02 ± 0.22
	C6	27.53 ± 4.34	1358	0.68 ± 0.12	11.64 ± 2.66	1919	0.58 ± 0.13
	C8	33.852 ± 5.87	1384	0.55 ± 0.09	5.11 ± 1.40	3868	0.26 ± 0.07

[a] Mean percentage eggs: mean percentage of eggs laid on morinda or acidic food; N: total number of eggs; C6: hexanoic acid; C8: octanoic acid; for each treatment 10 groups of adults were used as experimental repeats; S.E: standard error. Preference index: percentage observed/percentage expected (avoidance when less than 1, preference when superior to 1).

food. We thus established on the same plate two compartments separated by a stripe of paper (see Fig. 1B). One compartment (80% of the surface) was filled with control medium and the smaller one with experimental food.

Statistical Analysis

A typical experiment lasted 4 consecutive days. The female's age (4 to 7 days) corresponded to the maximum egg production in Drosophila (R'Kha *et al.*, 1997). The choice was estimated by considering the proportion of eggs laid on morinda or acidic medium: more than 50% (experiment with two egg-laying plates) or more than 20% (small cage with a single egg-laying plates) meaning a preference, and a lower proportion an avoidance. Some behavioral indices were used to characterize oviposition choice (see Results). For statistical analyses, percentages were arcsin square root transformed (angular transformation). No systematic change in oviposition choice was evidenced according to flies' age. For the sake of simplicity, the numbers of eggs obtained over successive days from the same groups of flies were added to produce a single value. Ten repeats (10 groups of flies) of each genotype were studied and averaged to characterize the oviposition behavior of each genotype (species and hybrids).

RESULTS

Influence of the Experimental Technique on Oviposition Choice: Behavior of the Four Species Towards Morinda Fruit

The four species were compared for their oviposition behavior versus morinda (Table I). With two plate cages, the species were clearly contrasted, in agreement with their ecological characteristics. *D. sechellia*, which breeds on morinda, laid more than 98% of its eggs on this fruit. The 3 other species, which avoid morinda in nature and are extremely sensitive to its toxicity, exhibited an absolute avoidance: no egg on morinda but more than 12,000 on laboratory food.

Such a clear-cut discrimination was unexpected in *D. mauritiana* because a previous work (Moreteau *et al.*, 1994) showed that this species exhibited a preference for morinda, when this fruit was spread on the surface of laboratory food on a single egg laying plate. We used a similar method (Fig. 1B) for testing the behavior when normal food and morinda were in close contact. The results were strikingly different for two species (Table I). We confirmed the unexpected behavior of *D. mauritiana* and its preference for morinda under such conditions, and *D. melanogaster* exhibited a similar preference. By contrast, *D. sechellia* and *D. simulans* did not change their behavior. We verified that, when deposited on morinda, *D. sechellia* eggs

were able to hatch (hatching percent of 84.4 for 300 eggs exposed); for the three other species, all embryos were killed.

These experiments suggest that the two methods do not measure exactly the same behavior. With two plates technique, the females are obliged to walk a few cm for moving from one plate to the other one. Some perception at distance seems to be involved, since morinda has a strong smell. The oviposition response may thus be described as either an attraction or a repulsion. The role of olfactory perception is certainly less important when the two substrates are in close contract, and oviposition is likely to depend more on gustatory receptors. In *D. sechellia*, these receptors provide a positive, preference signal and, in *D. simulans* a negative, avoidance one. In these two species, attraction and contact preference, or repulsion and contact avoidance, are positively correlated and act in synergy. Such is not the case in *D. mauritiana* and *D. melanogaster*, which exhibit a contact preference but a distance repulsion for morinda.

Oviposition Behavior Versus Aliphatic Acids: Influence of the Experimental Technique in the Four Species

Similar experiments were done with the laboratory medium supplemented with hexanoic or octanoic acid (Table I). After some preliminary assays, we choose a concentration of 0.5% of either acid, since it elicited a clear oviposition choice (see also Table II). In all cases *D. sechellia* and *D. simulans* exhibited a behavior qualitatively similar to that observed with morinda: preference in the former and avoidance in the latter. With the two plates technique, *D. sechellia* preference was significantly more pronounced for C6 than for C8. Similar preference indices were found for morinda and C6. With the single plate technique, the stronger preference for C6 was confirmed, but the preference for acidic food was less than for morinda. In *D. simulans*, avoidance of C6 and C8 was almost absolute with the two experimental techniques.

Results were more complex with the two other species. In all cases a preference for normal food was observed as indicated by indices less than unity: the acids could not replace morinda. Significant differences were found between the two acids but in opposite directions according to the experimental procedure and also the species. In *D. mauritiana*, C8 was more strongly avoided than C6 (p<0.01) with the two plates technique, but no difference (p = 0.15) was found with a single plate. An opposite trend seemed to occur in *D. melanogaster*: no difference with the two plates technique, and a stronger avoidance of C8 with the single plate technique.

From all these observations we may draw two practical conclusions: for genetic analyses, it will be better to use *D. simulans* as a parental species, and better to use the two plates technique, as presented in the next section.

Oviposition Behavior Versus Aliphatic Acids: Genetic Analyses with Interspecific Hybrids

We investigated the parental species, *D. sechellia*, and *D. simulans*, the F1 and the backcrosses. For each acid, three concentrations were compared, and for each genotype 10 groups of adults were used as experimental repeats. Basic data, after angular transformation, are given in Table II, and comparisons are made with a neutral behavior i.e., equal numbers of eggs on each plate. Numerous significant differences are also evidenced between hexanoic and octanoic acid.

The whole data set was submitted to ANOVA (Table III), and all the direct effects and all interactions were highly significant, illustrating the complexity of the behavioral responses. Genotypes explained 54% of the total variability, the quantity of acid 16%, and the nature of acid 5%. The strongest interaction was between dose and genotype. All these results needed a more precise description with help of Fig. 2 and 3.

The parental species exhibited contrasted oviposition behaviors in agreement with Table I. In *D. sechellia*, a clear preference was always found for the acidic food, the preference increased with the amount of acid, and C6 was always more attractive than C8 (Table II, Fig. 2 and 3). *D. simulans* exhibited an opposite behavior, and was repelled by the acids (except by C6 at low concentrations). This effect increased with the concentration, leading to a complete avoidance of the food containing 0.5% of acid. Differences between acids were generally non significant (Table II), except for the lowest dose (0.1%) and in that case the deterrent effect of C8 was more efficient.

The behaviors of hybrid genotypes (F1 and backcrosses) were more complex and data varied

Table II. Proportion of Eggs Laid on Acidic Plate (Two Plates Technique) by Females of Various Genotypes[a]

Genotype	Concentration	Hexanoic acid		Octanoic acid		Acids comparison (C6–C8)
		Eggs laid	Behavior response	Eggs laid	Behavior response	
D. sechellia	0.10%	59.38 ± 1.09	P***	52.19 ± 1.01	P***	+7.19***
	0.30%	67.54 ± 1.58	P***	54.39 ± 2.03	P**	+13.15***
	0.50%	76.37 ± 1.36	P***	57.15 ± 3.48	P**	+19.22***
BCsech	0.10%	52.44 ± 1.65	P**	50.64 ± 1.41	P**	+1.8[NS]
	0.30%	50.68 ± 1.82	P*	47.80 ± 1.93	N[NS]	+2.88[NS]
	0.50%	46.95 ± 1.66	N[NS]	25.96 ± 1.87	A***	+20.99***
F1	0.10%	51.57 ± 1.37	P**	51.98 ± 1.01	P***	−0.41[NS]
	0.30%	48.52 ± 2.05	N[NS]	52.5 ± 0.97	P***	−3.98[NS]
	0.50%	46.54 ± 2.15	N[NS]	18.65 ± 2.48	A***	+27.89***
BCsim	0.10%	47.06 ± 0.96	P*	41.62 ± 0.50	A***	+5.44**
	0.30%	38.68 ± 1.08	A***	16.71 ± 3.04	A***	+21.97***
	0.50%	9.17 ± 2.07	A***	2.36 ± 0.47	A***	+6.81**
D. simulans	0.10%	46.39 ± 0.72	N[NS]	31.41 ± 0.72	A***	+14.98***
	0.30%	18.8 ± 1.10	A***	19.03 ± 0.81	A***	−0.5[NS]
	0.50%	0	A	0	A	0

[a] Data were arcsin transformed and neutrality corresponds to a value of 45. Statistical comparisons with 45 indicate either preference behavior (P), neutrality (N) or avoidance (A). In each case, 10 repeats (groups of flies) were made. BCsech: *sechellia* backcross, F1: hybrid, BCsim: *simulans* backcross. NS, nonsignificant. *p<0.05, **p<0.01, ***p<0.001.

according to the nature and the concentration of acid. F1 females on C6 exhibited a behavior close to neutrality: a slight attraction with 0.1%, and no discrimination with higher doses. The concentration effect (Fig. 3) was not significant. With C8, the concentration effect was highly significant, changing from a slight preference with low doses to a clear avoidance with 0.5%. Dominance relationships were also highly variable and deserved a specific analysis (Table IV). With 0.1% C6, the F1 was always close to the mid-parent value and not significantly different, whereas with 0.3 and 0.5% significant differences were found, with a dominance of the characteristics of *D. sechellia*. With C8, on the other hand, significant dominance relationships were evidenced in each case, but changed according to concentration. For the two low concentrations (0.1 and 0.3%) the F1 behaved exactly like *D. sechellia*, whose properties appeared as fully dominant. With the highest concentration, however, F1 females avoided the acidic plate, in a way quite similar to the *D. simulans* parent, whose properties were dominant under these conditions.

Backcross genotypes were somehow intermediate between F1 and parental species, but again with variations according to experimental conditions. With C6, BCsech females were always very

Table III. Result of ANOVA on Oviposition Behavior According to Genotype (*D. sechellia*, *D. simulans*, F1, and Backcrosses) and Doses of Each Acid[a]

Source of variation	df	Mean square	F	Variability explained
Acid (1)	1	6470.95	246.80***	5%
Dose (2)	2	10325.11	393.80***	16%
Genotype (3)	4	17083.73	651.56***	54%
1 × 2	2	610.44	23.28***	1%
2 × 3	8	2165.44	82.60***	14%
1 × 3	4	165.35	6.31***	0.5%
1 × 2 × 3	8	608.09	23.19***	4%
Residual	270	26.22		5.5%

[a] Data were arcsin square root transformed. df: degrees of freedom, F: variance ratio. ***p<0.001.

close to F1 and no significant variation was evidenced among increasing doses. With C8, a significant concentration effect was observed, again quite similar to that found in F1, with a reversal from preference to avoidance.

BCsim females exhibited with both acids strong behavioral changes according to concentration. With low concentrations oviposition behavior was intermediate between that of the F1 and of the parental species. With higher concentrations (0.3 and 0.5% of C8 and 0.5% of C6) the behavior was
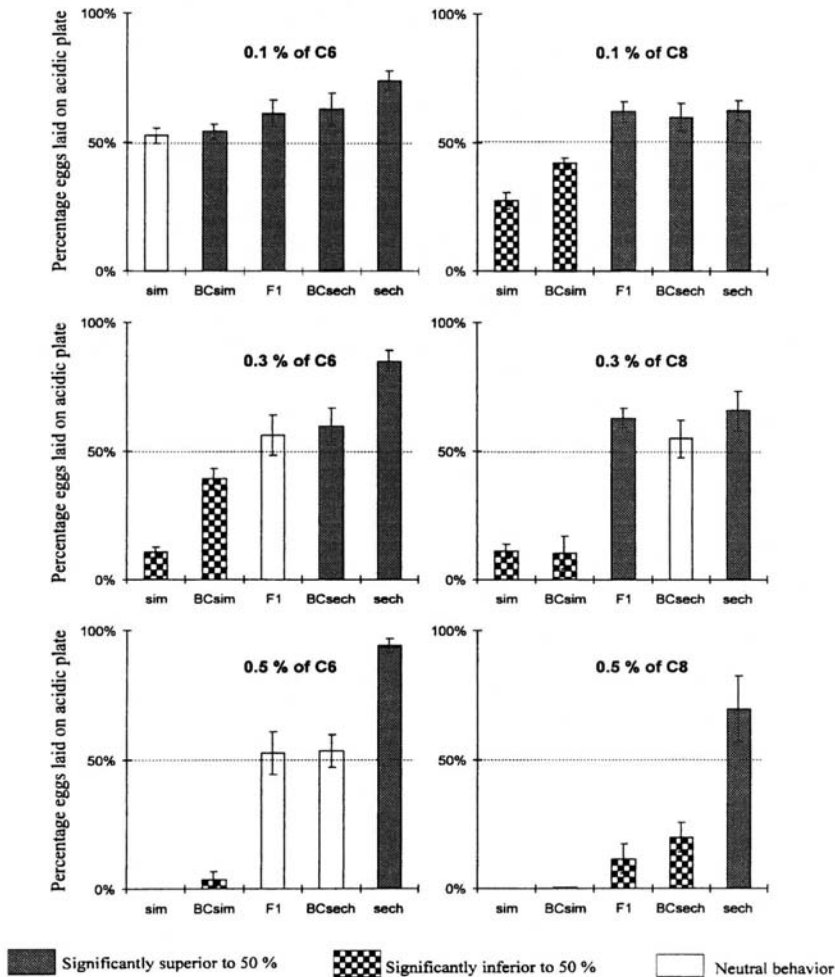
Fig. 2. Results of oviposition behavior according to the nature, concentration of acid, and genotype. The 50% value corresponds to a neutral behavior; vertical bars indicate confidence interval. sim: *D. simulans*, BCsim: backcross with *simulans*, F1: interspecific hybrid (*sechellia* males × *simulans* females), BCsech: backcross with *sechellia*, sech: *D. sechellia*.

more similar to that of the *D. simulans* parent, showing a dominance relationship.

DISCUSSION AND CONCLUSIONS

Behavioral investigations on Drosophila are often very sensitive to experimental procedure (Ehrman and Parson, 1976). A previous work (R'Kha *et al.*, 1991), in which a piece of morinda was sprayed on the surface of laboratory food, clearly showed an avoidance behavior by *D. simulans* and a preference of *D. sechellia*. The same method evidenced however an abnormal behavior in *D. mauritiana* (Moreteau *et al.*, 1994): females of that species preferred to oviposit on morinda, in

spite of the fact that all embryos were killed by the toxins. We confirmed this observation on *D. mauritiana* and found the same abnormal behavior in *D. melanogaster*. When however, morinda and normal food were put in different egg-laying plates separated by a short distance, we observed a marked preference in *D. sechellia*, but an absolute avoidance in the 3 other species, in better agreement with ecological data.

As argued in the result section, such observations suggest that the two methods measure different behaviors. In a recent publication, Foster and Harris (1997) reviewed the methods used for protecting crops by manipulating the behavior of pest insects. These methods were divided into two cat-

egories: those that manipulated behavior at a short distance, less than 1cm, and involved non volatile chemicals, and those which manipulated behavior over a large distance, using either volatile chemical or visual or auditory stimuli. In the present work, we have evidenced a clear cut distance effect, but with the same chemical product. A simple hypothesis is that different receptors were involved in different behavioral sequences. When two egg-laying plates are available, females are obliged to walk a few cm to reach the other plate, and some volatile signal should be involved, perceived by olfactory receptors on the antennae and maxillary palps (Carlson, 1996). Indeed we already know that adults of *D. sechellia* are attracted by morinda traps while those of *D. simulans* are repelled (R'Kha *et al.*, 1991; Legal *et al.*, 1992). A similar phenomenon was described with hexanoic acid (Higa and Futuyama, 1993).

When the two possible resources are in close contact, olfactory attraction, or repulsion, will be less efficient. The result observed could be mediated by contact chemoreceptors, located on the proboscis and the tarsi (Morita and Shiraishi, 1985). These receptors provide, as expected, a positive oviposition signal in *D. sechellia*, and a negative deterrent signal in *D. simulans*. It remains however surprising that *D. mauritiana* and *D. melanogaster* exhibited a behavior similar to that of *D. sechellia*. In a previous work (Moreteau *et al.*, 1994), it was suggested that the performance observed in *D. mauritiana* could be mediated by some undefined compounds found in morinda, but not by aliphatic acids. Our new data have shown that these two species avoided the acidic food with both experimental techniques: the aliphatic acids cannot replace morinda and do not elicit a positive egg laying signal. Further investigations are obviously needed for analyzing the response to morinda.

The genetic divergence between *D. sechellia* and *D. simulans*, with respect to aliphatic acids, was therefore analyzed with separate laying plates, the results of which are closer to the natural ecological situation.

For both acids we found that 0.1% was a low concentration providing only slight differences between parental species. Only the highest concentration (0.5%) led to clear cut different behaviors, i.e., a complete avoidance in *D. simulans* and an almost complete preference in *D. sechellia*. The higher efficiency of C6 on *D. sechellia* might be due to its
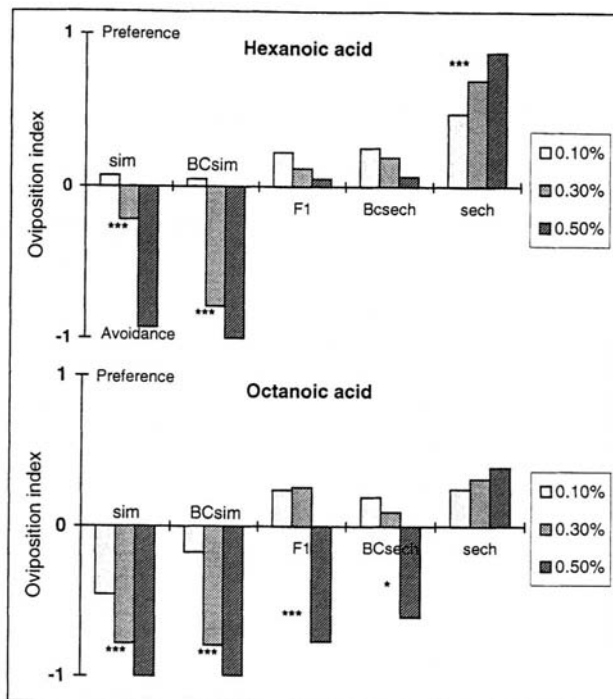

Fig. 3. Summary of the dose effect upon oviposition behavior of the various genotypes. Oviposition index is the difference in the number of eggs laid on acidic and normal plate, divided by total egg number. Neutral behavior corresponds to zero, positive values to preference, negative values to avoidance. Same symbols for genotypes as in Fig. 2. For each genotype the dose effect was checked with ANOVA (results not shown). Significant effects are marked on the graphs. *$p < 0.05$, ***$p < 0.001$.

higher volatility, providing a more efficient attraction. In natural morinda, the amounts of C6 and C8 are quite variable among fruits, and the quantity of C8 (352 ± 210 µg/g) is always higher than C6 (65 ± 80 µg/g) (Legal *et al.*, 1994). In the present work, we were obliged to use much higher concentrations than those found in morinda to elicit a similar behavioral response. Such a difference may be explained by the fact that a large proportion of the acid combines with the instant Drosophila medium, so that the efficient amount of free acid is in fact much less than the concentration really introduced in the food. In agreement with this hypothesis, we may recall that a high proportion of the sensitive *D. simulans* adults were killed when one laying plate was filled with morinda (3g). No abnormal mortality was observed with acidic medium even with the highest concentration.

Table IV. Comparison of Oviposition Behavior of F1 Females with Mid-parent Value (MP)[a]

Acid	Dose	Mid-parent	F1 hybrid	Difference (MP-F1)	t test (df = 18)	Dominance
C6	0.1%	52.89 ± 0.65	51.56 ± 1.37	+1.32 ± 1.52	0.87NS	No
C6	0.3%	43.17 ± 0.89	48.52 ± 2.05	−5.35 ± 2.34	2.39*	*sechellia*
C6	0.5%	38.18 ± 0.68	46.53 ± 0.30	−8.18 ± 2.21	3.7**	*sechellia*
C8	0.1%	41.80 ± 0.70	51.98 ± 1.01	−10.17 ± 1.23	8.26***	*sechellia*
C8	0.3%	36.71 ± 0.96	52.50 ± 0.97	−15.79 ± 1.38	11.55***	*sechellia*
C8	0.5%	28.57 ± 0.68	18.65 ± 2.48	+9.92 ± 2.57	3.86**	*simulans*

[a] Values are the proportion of eggs laid on acidic plate (transformed values ± standard error). Signed differences are compared to zero with a Student's t test. C6: hexanoic acid, C8: octanoic acid, df: degrees of freedom. NS: nonsignificant. *p<0.05, **p<0.01, ***p<0.001.

A basic problem in behavior genetics is the number and function of the genes determining a phenotype. A quite simple interpretation of the data would have been possible if only one concentration of only one acid had been used. Such was the case with natural morinda (R'Kha *et al.*, 1991). Our investigations were however more extensive since two chemicals have been used and, for each, three concentrations. Major differences were found between genotypes (Table II). Hexanoic acid was generally more efficient than C8 in eliciting a preference, while octanoic acid was more efficient than C6 when eliciting an avoidance. This conclusion was valid for parental species and hybrid genotypes. Since C6 is more volatile than C8, and since two types of receptors seem to be involved (olfaction and chemical contact) such differences might be explained by a differential use of different receptors.

Significant concentration effects were observed in most genotypes (Fig. 3) with three exceptions: *D. sechellia* with C8; F1 and BCsech with C6. An increasing preference or avoidance when the signal strength increased was a logical and expected response, found in parental species. It was however surprising to observe a complete behavior reversal from preference to avoidance in 3 cases: F1 and BCsech with C8 and BCsim with C6. Comparing hybrids to mid-parent showed that a dominance relationship could be also completely changed according to the dose. The clearest example was F1 females with C8, showing a strong dominance of *D. sechellia* at low doses (0.1 and 0.3%) but a reverse dominance of *D. simulans* at the highest dose (0.5%). A plasticity of dominance was recently described for insecticide resistance according to technical procedures (Bourguet *et al.*,

1996), which was difficult to interpret according to Wright's physiological theory (Wright, 1977). Our data are still more difficult to explain in terms of function since the responsible gene(s) are not known and since different receptors, with different threshold values, may interact.

These observations are however interesting to discuss in terms of progressive adaptation. As argued previously (R'Kha *et al.*, 1997), we know the direction of evolution from an ancestral sensitive state (as in *D. simulans*) to a derived tolerant one as found in *D. sechellia*. The selection on toxic morinda, in the Seychelles, was certainly a progressive process, since the acids disappear from the ripe fruit during rotting (Legal *et al.*, 1992). A diversity of sensitive species may be bred from rotten natural morinda (Louis and David, 1986; David *et al.*, 1989). In nature, *D. sechellia* may have a temporal advantage since its tolerance permits an earlier colonization of the resource, which compensates its very low fecundity (Louis and David, 1986; R'Kha *et al.*, 1997). When behavioral oviposition variants were selected, it was certainly advantageous to them to be dominant on low concentrations, so that natural selection was more efficient. Of course, natural selection acted simultaneously on the resistance to C6 and C8 (Amlou *et al.*, 1997) and on the behavioral attraction of adults for C6 (Higa and Futuyama, 1993). Whether all these changes imply pleiotropic effects or multiple loci remains however an objective for further studies.

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