Genetica **88**: 129–136, 1993.

Genetics of food preference in *Drosophila sechellia*

I. Responses to food attractants

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Key words: *Drosophila sechellia*, host selection, *Morinda citrifolia*, olfactory response

Abstract

To reveal the genetic mechanism of host selection in a monophagous fruit fly *Drosophila sechellia*, olfactory responses and oviposition preferences of this species were compared with those of closely related polyphagous species, *D. simulans* and *D. melanogaster*. Adult flies of *D. sechellia* were strongly attracted to the ripe fruit of *Morinda citrifolia* which is known to be the sole breeding site of this species. They were also attracted to the odor of *n*-caproic acid which is contained in the ripe fruit of *M. citrifolia* and is presumably responsible for the characteristic odor of the fruit. In contrast, *D. simulans* and *D. melanogaster* showed a strong repulsion to *n*-caproic acid. In parallel with the olfactory responses, *D. sechellia* females laid eggs preferentially on a medium containing *n*-caproic acid, to which the other two species showed an aversion. Genetic analyses using the hybrid progeny between *D. sechellia* and *D. simulans* suggested that the species differences in these behaviors are controlled by gene(s) located on the second chromosome.

Introduction

Host selection of phytophagous insects has been receiving special attention in relation to the topic of sympatric speciation, which has been a controversial subject for many years. Maynard Smith (1966) pointed out that the initial step of sympatric speciation requires the establishment of a stable polymorphism between alleles that are responsible for adaptation to different ecological niches. This may be realized when population sizes in each niche are regulated separately, and disruptive selection is strong, although these conditions may be rarely satisfied in nature. However, in the cases of monophagous or oligophagous insects, those conditions are likely to be met, provided that mutations enable them to adapt to utilize separate host plants. Circumstantial evidence for sympatric speciation through host changes has been amply documented (e.g. Tauber & Tauber, 1989, and literature therein). Nevertheless, the genetic bases of host choice in these insects are poorly understood, though some genetic studies have been carried out with the host races of *Rhagoletis* (Feder *et al.*, 1990a, 1990b) and *Papillio* (Thompson *et al.*, 1990).

In *Drosophila*, Rice and Salt (1988, 1990) demonstrated that some degree of assortative mating could be developed in the laboratory by imposing a strong disruptive selection for habitat choice. This suggests that reproductive isolation may result from divergent habitat preferences. However, neither distinct host races nor sympatric sibling species that may indicate speciation by host shift seem to have been reported.

An interesting exception has been noticed in *Drosophila sechellia*, the newest member of the melanogaster subgroup described by Tsacas and Bächli (1981), from the Seychelles Islands. Unlike most of the other members of the subgroup, *D. sechellia* is known to breed exclusively on the ripe fruit of an arboreal Rubiaceae, *Morinda citrifolia* (Louis & David, 1986). Despite its obligatory monophagy in nature, *D. sechellia* is relatively easily raised in the laboratory using conventional *Drosophila* food. Moreover, interspecific hybrids can be obtained when *D. sechellia* is crossed to its sibling species, *D. melanogaster* and *D. simulans*, which have rich genetic resources (Lachais *et al.*, 1986). These properties of *D. sechellia* provide us a rare opportunity for investigating the genetic mechanisms for its food specialization.

R'kha, *et al.* (1991) pioneered the genetics of host selection in *D. sechellia*. They demonstrated that the fruit of *M. citrifolia* is toxic to *D. simulans*, but not to *D. sechellia*. By examining the survival on morinda of hybrids between the two species and that of backcross progeny, it was suggested that the tolerance for the toxic effect is controlled by a dominant gene. They also showed that attraction to morinda and oviposition on it are controlled by multiple factors. They concluded that at least three or four independent loci are involved in the host specialization of *D. sechellia*. Further investigation along this line would make an important contribution to our understanding of genetic mechanisms of host shift in herbivorous insects.

In the present paper, we will show that *D. sechellia* is attracted to the odor of several low fatty acids, which are putative attractants contained in the morinda fruit, and that females deposit eggs preferentially on the medium containing *n*-caproic acid. We also present evidence that these behaviors are controlled by a small number of major genic factors.

Materials and methods

Strains

Strains of *Drosophila sechellia* used in this study were five isofemale lines, SS51, SS52, SS77, SS78 and SS86, originally colleced by Ishiwa *et al.* on Plaslin island, the Seychelles in 1986. Isofemale lines of *D. simulans* (S352, S353, S354, S355, S357) and *D. melanogaster* (M361, M402, M403, M404, M405) were derived from a collection at Oiso, Kanagawa Prefecture, Japan in 1979. Strains of *D. simulans* with dominant marker *Spread* (*Spd*, 2-0.4; Saito & Watanabe, 1986; Inoue *et al.*, 1988) and *Ultrabithorax* (*Ubx*, 3-58.8; Barker, J. S. F., personal communication to Watanabe, T. K.) were provided by Drosophila stock center at the National Institute of Genetics, Misima. All strains were kept on a corn meal-yeast-glucose medium at 25 °C.

Odorants

Ripe fruit of *Morinda citrifolia* (referred to as morinda in this paper) was collected at the beaches of Ishigaki Island and Taketomi Island, both located in Okinawa Prefecture, Japan. The fruit was stored at -20 °C and was thawed at room temperature before use. Juice was obtained by squeezing the thawed fruit.

All chemicals used as stimulants were of reagent grade. Lactic acid, *n*-butyric acid, *n*-valeric acid, isovaleric acid and *n*-caproic acid were purchased from Wako Pure Chemicals Inc., 3-pentanone was purchased from Tokyo Kasei Co., and salicylaldehyde from Kanto Kasei Co.

Trap assays

Trap assays were conducted in a cage made of a plastic container (12 × 11 × 9 cm) with a tight-fitting lid. The lid has an opening (4 × 5 cm) in the center covered with fine wire meshes for ventilation. Two 30 ml glass flasks were placed in opposite corners as traps; one trap contained 20 ml of odorant solution, and another one contained the same amount of distilled water as a control. Triton-X 100 were added to both traps to 0.05% to drown flies.

Flies two to four days old were sexed without anesthesia, and approximately 100 flies of either sex were introduced into the cage. It was kept in a dark ventilated chamber for 12 h, and most of the flies chamber fell into either one of the traps during this period. The number of trapped flies was scored for each trap. The proportion of the flies trapped in the odorant-containing trap to total flies trapped was arc-sine transformed into a degree and was referred to as a response index.

Oviposition-site preference

Oviposition-site preference was tested by using the same apparatus as the trap assay, but with traps substituted for by plastic vial caps containing media. Tests were carried out on a morinda-agar medium and a yeast medium impregnated with 0.5% *n*-caproic acid. The morinda-agar medium was made of 95% morinda juice and 0.5% agar. The yeast medium consisted of 20% brewer's yeast, 0.8% agar and 5% sucrose. Ten male and ten female flies of four to seven days old were added into each cage and left in a ventilated chamber in the dark at 25 °C. Flies were removed after 24 h, and the number of eggs laid on each medium was scored.

Results

Response to morinda and various chemicals

Responses of the three species of *Drosophila* to morinda juice were tested by the trap assay. The results are shown in Table 1. Strains used were SS86 (*D. sechellia*), S357 (*D. simulans*) and M402 (*D. melanogaster*). *D. sechellia* was attracted to the trap containing morinda juice, i.e. their response index was significantly larger than 45, which indicates a neutral response (t-test, $p<0.01$). Although *D. melanogaster* was also attracted to the morinda juice, *D. sechellia's* attraction was significantly stronger than that of *D. melanogaster* (t-test, $p<0.01$).

Responses of the three species to the odorants that are known to be attractants (1% lactic acid and 1/32% 3-pentanone; Fuyama, 1976, 1979) and a repellent (1/32% salicylaldehyde; Kikuchi, 1973) to *D. melanogaster* was also tested. All the three species showed attraction to lactic acid and repulsion to salicylaldehyde. However, 3-pentanone repelled *D. sechellia* while it attracted *D. melanogaster* and *D. simulans*.

Table 1. Responses of three species of *Drosophila* to various odorants. Value represents the mean response index (± S.E.) of ten replicate tests, five per sex.

Odorants	Species		
	Drosophila sechellua	*D. simulans*	*D. melanogaster*
Morinda juice	77.4*** (± 1.1)	49.9 (± 2.5)	57.1* (± 4.1)
1% Lactic acid	54.7* (± 4.5)	53.5* (± 3.0)	54.6* (± 3.2)
1/32% 3-Pentanone	39.3† (± 2.8)	53.9** (± 2.3)	62.3*** (± 1.4)
1/32% Salicyl-aldehyde	10.9 ††† (± 2.1)	5.8 ††† (± 2.0)	10.0 ††† (± 2.7)

* = P < .05; ** = P < .01; *** = P < .005: Significantly larger than 45; † = P < .05; †† = P < .01; ††† = P < .005: Significantly smaller than 45.

Table 2. Responses of three species of *Drosophila* to fatty acids. Value represents mean response index (± S.E.) of ten replicate tests, five per sex.

Fatty acids	Species		
	Drosophila sechellia	*D. simulans*	*D. melanogaster*
1% *n*-Butyric acid	57.4** (± 3.1)	25.4†† (± 1.4)	47.2** (± 0.6)
1% *n*-Valeric acid	48.2** (± 1.1)	11.4††† (± 2.3)	24.4††† (± 3.6)
1% Isovaleric acid	46.4 (± 1.8)	31.1†† (± 2.1)	48.9* (± 1.4)
1% *n*-Caproic acid	66.1*** (± 2.0)	5.8††† (± 1.3)	10.0††† (± 1.0)

* = P< . 05; ** = P < .01; *** = P < .005: Significantly larger than 45; † = P < .05; †† = P < .01; ††† = P < .005: Significantly smaller than 45.

Attractant for D. sechellia

Lachais and Tsacas (1983) report that, in the field, *D. sechellia* is attracted by the 'smell like decaying cheese' of morinda. A gas chromatography analysis of ethanolic extracts of ripe morinda fruit revealed several fatty acids of low molecular weight such as *n*-butyric acid, isobutyric acid, *n*-valeric acid, isovaleric acid, *n*-caproic acid, isocaproic acid and capryl acid. These fatty acids are presumably responsible for the characteristic odor emitted from morinda, and thus may be constituents of food attractant for *D. sechellia*. Therefore, responses of *D. sechellia*, *D. simulans*, and *D. melanogaster* to 1% of *n*-butyric acid, *n*-valeric acid, isovaleric acid and *n*-caproic acid were examined by the trap test. Strains used were SS86 (*D. sechellia*), S357 (*D. simulans*) and M402 (*D. melanogaster*). The results are shown in Table 2. *D. sechellia* was attracted to all the fatty acids except isovaleric acid, to which it showed a neutral response. *D. simulans* was repelled by all the fatty acids and *D. melanogaster* was slightly but significantly attracted by *n*-butyric acid and isovaleric acid, but was repelled by *n*-valeric acid and *n*-caproic acid.

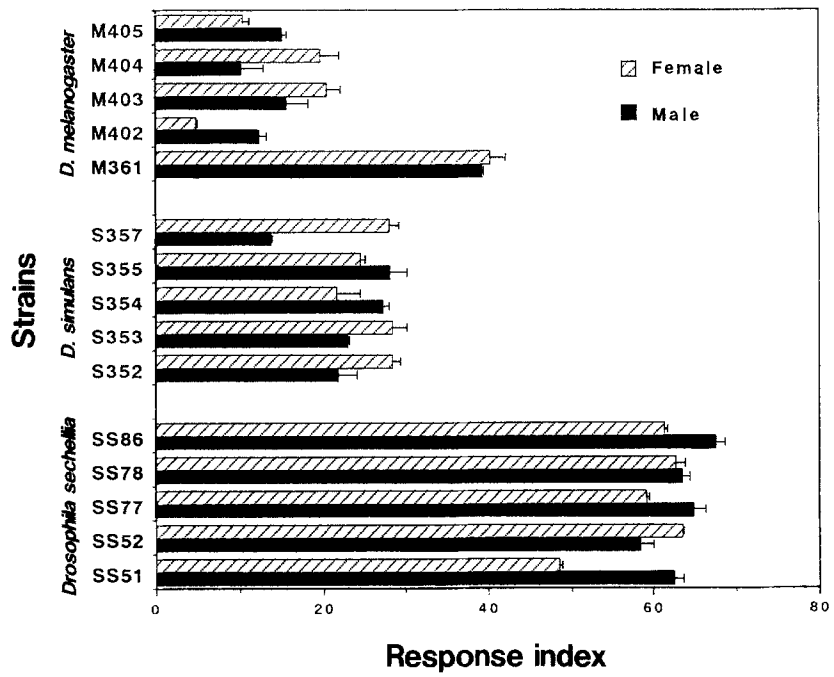

Fig. 1. Responses of the strains of *Drosophila sechellia*, *D. simulans* and *D. melanogaster* to 1/2% *n*-caproic acid. Bars represent the average response index of ten replicate tests (five for each sex); vertical lines show the range of standard errors.

Response to n-caproic acid

Since the responses to *n*-caproic acid were found to be most contrasting between *D. sechellia* and the other two sibling species, we used *n*-caproic acid for further investigation. To test whether the response to *n*-caproic acid differs by sex or strain, responses to 1/2% *n*-caproic acid were examined for five isofemale strains each of three species (Fig. 1). An analysis of variance (ANOVA) showed that most of the variance was due to the difference among species, but the inter-strain difference was also found to be significant (Table 3). ANOVAs carried out for the three species separately showed that variance among strains was significant only in *D. melanogaster*. This was due to the strain M361 that showed neither significant repulsion nor attraction to 1/2% *n*-caproic acid (Fig. 1).

Table 3. ANOVA for olfactory responses to *n*-caproic acid.

Source of variance	S.S.	d.f.	M.S.	F
Sex	17.9	1	17.9	0.6
Species	36808.8	2	18404.4	1723.3**
Sex × Species	252.7	2	126.3	1.7
Strain/Species	3553.6	12	296.1	10.7*
Sex × Strain	886.1	12	73.8	2.7
Error	1663.6	60	27.6	

* = P < 0.01; ** = P < 0.001

Responses of the three species to *n*-caproic acid of various concentrations were investigated by the trap assay, applying *n*-caproic acid in a binary dilution step from 1% to 1/256%. Strains used were SS86 (*D. sechellia*), S357 (*D. simulans*) and M402 (*D. melanogaster*). The dose-response curves are shown in Fig. 2. Experiments were carried out for each sex, but since no sexual difference was found to be significant, data obtained with both sexes were pooled. All the three species showed a weak attraction to *n*-caproic acid at low concentrations. *D. melanogaster* showed a repulsion at concentrations over 1/64%, and *D. simulans* showed a repulsion at concentrations over 1/8%. *D. sechellia* however, was attracted to every concentration tested.

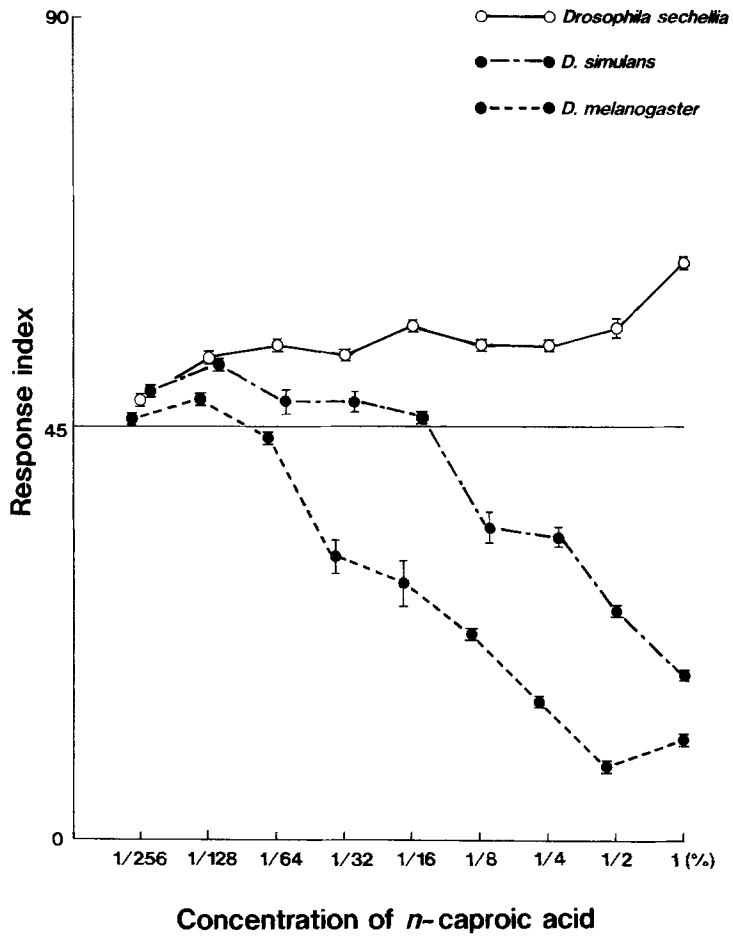

Fig. 2. Dose-response curves of *Drosophia sechellia*, *D. simulans* and *D. melanogaster* to *n*-caproic acid. Each point represents the average response index of ten replicate tests (five for each sex); the vertical lines show the range of standard errors.

Oviposition-site preference

The results of the test for the choice of oviposition sites are shown in Table 4. Strains used were SS86 (*D. sechellia*), S357 (*D. simulans*) and M402 (*D. melanogaster*). When flies were offered a choice between a medium containing morinda juice and a yeast-agar medium, more than 98% of *D. sechellia* eggs were laid on the morinda medium (Table 4a). In contrast, *D. simulans* and *D. melanogaster* showed a complete aversion to the morinda medium and laid all of their eggs on the yeast-agar medium. When the same test was carried out with a medium impregnated with 1/2% *n*-caproic acid, essentially the same results were obtained. *D. sechellia* laid most of their eggs on the *n*-caproic acid medium while the other two species showed strong aversions to it (Table 4b).

Genetic analysis

To know the genetic basis of the differences found between *D. sechellia* and the other two species in their responses to morinda juice or *n*-caproic acid, F_1 hybrids between *D. sechellia* and *D. simulans*, and backcross progeny were examined for olfactory responses to *n*-caproic acid. Strains used were SS86 (*D. sechellia*) and S357 (*D. simulans*). As shown in Table 5, the F_1 hybrids between *D. sechellia* and *D. simulans* showed the same level of repulsion to

Table 4. Oviposition preference of three species of *Drosophila* for morinda medium (*a*) and yeast medium impregnated with 1/2% *n*-caproic acid (*b*). Value represents the number of eggs (%) laid on each medium in five replicate tests.

a.

Medium	Species		
	Drosophila sechellia	*D. simulans*	*D. melanogaster*
Morinda medium	52 (98.1%)	0 (0%)	0 (0%)
Control (Yeast medium)	1 (1.9%)	702 (100%)	679 (100%)

b.

Medium	Species		
	Drosophila sechellia	*D. simulans*	*D. melanogaster*
1/2% *n*-Caproic acid	20 (87.0%)	0 (0%)	1 (0.7%)
Control (Yeast medium)	3 (13.0%)	537 (100%)	141 (99.3%)

Table 5. Olfactory responses of *D. sechellia*, *D. simulans* and hybrid progeny to 1/2% n-caproic acid. Value represents mean attraction index (± S.E.) of five replicate tests except for a case indicated by #, in which replicates were three.

		Female	Male
Drosophila sechellia		65.6*** (± 2.1)	60.0*** (± 1.4)
D. simulans		24.8††† (± 2.3)	21.3††† (± 1.3)
F_1#	*D. sechellia* ♀ × *D. simulans* ♂	22.7††† (± 2.6)	27.6††† (± 0.7)
F_1	*D. simulans* ♀ × *D. sechellia* ♂	25.1††† (± 1.6)	22.1††† (± 2.7)
BC_1	F_1 ♀ × *D. sechellia* ♂	45.6 (± 1.2)	46.3 (± 1.5)

* = P < .05; ** = P < .01; *** = P < .005: Significantly larger than 45; † = P < .05; †† = P < .01; ††† = P < .005: Significantly smaller than 45.

n-caproic acid as *D. simulans* did. No substantial difference was found between the reciprocal crosses, indicating that factors located on the X-chromosomes are not involved. Because only a small number of F_1 progeny can be obtained from the cross between *D. sechellia* females and *D. simulans* males, and because all hybrid males are sterile in the reciprocal crosses, backcross is possible only between *D. sechellia* males and F_1 females. The response of BC_1 flies was in between that of *D. sechellia* and F_1 hybrids. These results indicate that the main genetic factor(s) responsible for the species difference are recessive and autosomal. The same conclusion was obtained from the oviposition test (data not shown).

Further genetic analyses were carried out using the visible dominant markers of *D. simulans*. Males of *D. sechellia* (SS86) were crossed with *D. simulans* females whose second and third chromosomes were marked with *Spd* and *Ubx*, respectively, to obtain fertile F_1 females, which were backcrossed to *D. sechellia* males. The resulting BC_1 flies were subjected to the trap assay. After the test, the genotypes of trapped flies were determined. In total, 1780 females and 1202 males were trapped in 20 replicate tests, and the number of flies collected from the respective traps were summarized according to their genotypes in Table 6a. To evaluate the effects of the second and third chromosomes on the response, a linear model fitting was performed on the data pooled for both sexes using the CATMOD procedure of the SAS package (SAS Institute, 1985). A maximum-likelihood ANOVA for the estimated parameters demonstrated a highly significant contribution of the second chromosome (Table 6b), indicating that the factor(s) responsible for the differences between *D. sechellia* and *D. simulans* is located on the second chromosome.

Table 6. Responses of BC_1 progeny to 1/2% *n*-caproic acid. BC_1 progeny were obtained from a cross between *Drosophila sechellia* males and the F_1 hybrid females of *D. sechellia* and *D. simulans*, whose second chromosome marked by *Spd* and the third chromosome marked by *Ubx*. *a*: Number of individuals (%) trapped in twenty replicate tests, ten for each sex. *b*: Maximum-likelihood ANOVA for the linear model fitting of the olfactory responses of BC_1 progeny.

a.

	Phenotype			
Traps	*Spd; Ubx*	*Spd; +*	*+; Ubx*	*+; +*
n-Caproic acid	243 (37.9%)	255 (34.0%)	289 (54.9%)	668 (62.7%)
Control	398 (62.1%)	494 (66.0%)	237 (45.1%)	398 (37.3%)

b.

Source	DF	Chi-Square	Probability
Intercept	1	7.91	0.0049
Second chromosome	1	144.17	0.0000
Third chromosome	1	0.89	0.3452
Second chromosome × Third chromosome	1	9.97	0.0016

Discussion

If a phytophagous insect is to exploit a novel host plant, various adaptive changes in behavior and physiology must be required for its host utilization; adult females need to recognize the new host plant and deposit eggs on it, and larvae must utilize it for nutrition, and very often must cope with the host's defense mechanisms. How many gene loci are involved in these changes is a crucial question in determining readiness of host race formation which may eventually lead to speciation. Although a number of genetic studies of host races of various groups of insect have been made to answer this question (Tauber & Tauber, 1989, and literature therein; Feder *et al.*, 1990a, 1990b; Thompson *et al.*, 1990; Wood & Keese, 1990; Wood *et al.*, 1990), the genetic basis of host switch is still far from clear.

Drosophila sechellia is a unique species for its monophagy. Several lines of evidence suggest that this species evolved probably around half a million years ago (Lachaise *et al.*, 1986; Coyne & Kreitman, 1986; Cariou, 1987). Considering that most members of the melanogaster subgroup to which *D. sechellia* belongs are polyphagous, it is conceivable that exploitation of the specialized food resource by *D. sechellia* played an important role in its speciation event. Closely related to *D. melanogaster* and *D. simulans*, *D. sechellia* would be a suitable model for investigating the genetic mechanism of host selection in insects.

The present study has shown that adults of *D. sechellia* are attracted to the odors of several low fatty acids, especially that of *n*-caproic acid, as well as to the ripe fruit of *Morinda citrifolia*. *D. sechellia* is not defective in sensing or avoiding aversive odor, since it showed a repulsion for salicylaldehyde as strongly as the other species did (Table 1). Those fatty acids are contained in the ripe fruit of *M. citrifolia* (Higa & Fuyama, 1990). *D. sechellia* and the other two sibling species showed strong contrast in response similarly toward *n*-caproic acid and the morinda fruit. These results strongly support that *n*-caproic acid is involved in food recognition by *D. sechellia*. This study has also shown that females lay eggs preferentially on the *n*-caproic acid impregnated medium. It remains to be determined whether this behavior is a result from attraction to the medium or if *n*-caproic acid acts as an oviposition stimulant. If the former is the case, then the two major behaviors necessary for female parents, detection of breeding site and oviposition on it, may be controlled by a common genetic system. It has been reported that olfactory preferences affect oviposition in *Drosophila* (Ayala & Ayala, 1969; Lofdahl, 1986).

R'kha *et al.* (1991) suggested the effects of polygenes on the responses. Their genetic analyses suggested that separate genes are responsible for behavioral response to morinda fruit and oviposition preference. Contact chemical and/or tactile stimuli elicited from morinda are likely to affect the oviposition behavior. Many volatile substances contained in the morinda fruit other than fatty acid could affect the attraction or repulsion behavior. The responses of flies to qualitatively different stimuli would be controlled by different sets of genes, the overall effects of which may be discernible as a polygenic trait.

In our study, the responses to *n*-caproic acid of F_1 hybrids between *D. sechellia* and *D. simulans*, and those of BC_1 progeny suggest that the behavioral differences between the two species can be explained by a small number of loci with attraction being recessive to repulsion. The major factor(s) seems to be localized on the second chromosome. Attraction by *D. sechellia* and repulsion by *D. simulans* could be independently controlled by separate loci. To explore this possibility, we are currently mapping the factor(s).

Acknowledgements

We thank Professors O. Kitagawa, Y. Tobari, and Dr. T. Aotsuka for their encouragement and valuable advice, Professor M. Sato and Dr. A. Hiraoka for gas chromatography analysis of fatty acids, Dr. T. K. Watanabe for providing us with the marker strains of *Drosophila simulans*, and Drs., T. Asami and M. Yamamoto for the critical reading of the manuscript. This work was partly supported by a grant (No. 62041032 to S. Ishiwa.) from the Ministry of Education, Science and Culture of Japan.

References

Ayala, F. J. & M. Ayala, 1969. Oviposition preference in *Drosophila melanogaster*. Drosophila Inf. Serv. 44: 120.

Cariou, M. L., 1987. Biochemical phylogeny of the eight species in the *Drosophila melanogaster* subgroup including *D. sechellia* and *D. orena*. Genet. Res. Camb. 50: 181-185.

Coyne, J. A. & M. Kreitman, 1986. Evolutionary genetics of sibling species, *Drosophila simulans* and *D. sechellia*. Evolution. 40: 673-691.

Feder, J. L., C. A. Chicote & G. L. Bush, 1990a. The geographic differentiation between host associated populations of *Rhagoletis pomonella* (Diptera: Tephritidae) in the eastern United Stated and Canada. Evolution. 44: 570-594.

Feder, J. L., C. A. Chicote & G. L. Bush, 1990b. Regional, local and microgeographical allele frequency variation between apple and hawthorn populations of *Rhagoletis pomonella* in western Michigan. Evolution. 44: 595-608.

Fuyama, Y., 1976. Behavior genetics of olfactory responses in *Drosophila*. I. Olfactometry and strain differences in *Drosophila melanogaster*. Behav. Genet. 8: 399-410.

Fuyama, Y., 1978. Behavior genetics of olfactory responses in *Drosophila* II. An odorant-specific variant in a natural population of *Drosophila melanogaster*. Behav. Genet. 8: 399-441.

Higa, I. & Y. Fuyama, 1991. Genetics of food preference in *Drosophila sechellia* III. (Abstract). Jpn. J. Genet. 66: 795.

Inoue, Y. H., T. Taira & M. T. Yamamoto, 1988. Genetics of an unstable *white* mutant in *Drosophila simulans*: Reversion, suppression and somatic instability. Genetics. 119: 903-912.

Kikuchi, T., 1973. Genetic alteration of olfactory functions in *Drosophila melanogaster*. Jpn. J. Genet. 48: 105-118.

Lachaise, D., J. R. David, F. Lemunier & L. Tsacas, 1986. The reproductive relationships of *Drosophila sechellia* with *D. mauritiana*, *D. simulans* and *D. melanogaster* from the Afrotropical region. Evolution. 40: 262-271.

Lachais, D. & L. Tsacas, 1983. Breeding-sites in tropical African Drosophilids, pp. 221-332. In: Ashburner, M., H. L. Carson & J. N. Thompson, Jr. (eds.), The Genetics and Biology of *Drosophila*. vol. 3d, Academic Press, London.

Lemunier, F. & M. Ashburner, 1984. Relationships within the melanogaster species subgroup of the genus *Drosophila* (*Sophophora*). IV. The Chromosomes of two new species. Chromosoma. 89: 343-351.

Lofdahl, K. L., 1986. A genetic anaysis of habitat selection in the cactophilic species, *Drosophila mojavensis*. pp. 153-162. In: Huettel. M. D. (ed.), Evolutionary Genetics of Invertabrate Behavior. Plenum Press, N.Y.

Louis, J. & J. R. David, 1986. Ecological specialization in the *Drosophila melanogaster* species subgroup: A case study of *D. sechellia*. Ecol. Genet. 7: 215-229.

Maynard Smith, J., 1966. Sympatric speciation. Am. Nat. 100: 637-651.

Rice, W. R. & G. W. Salt, 1988. Speciation via disruptive selection on habitat preference: Experimental evidence. Am. Nat. 105: 83-86.

Rice, W. R. & G. W. Salt, 1990. The evolution of reproductive isolation as correlated character under sympatric conditions: Experimental evidence. Evolution. 44: 1140-1152.

R'Kha, S., P. Capy & J. R. David, 1991. Host-plant specialization in the *Drosophila melanogaster* species complex: A physiological, behavioral, and genetical analysis. Proc. Natl. Acad. Sci. USA 88: 1835-1839.

Saito, K. & T. Watanabe, 1986. New mutants in Drosophila simulans. (Abstract). Jpn. J. Genet. 61: 611.

SAS Institute, 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Inst., Inc., Cary, NC.

Tauber, C. A. & M. J. Tauber, 1989. Sympatric speciation in insects: Perception and perspective. pp. 307-344. In: Otte, D. and J. A. Endler (eds.), Speciation and Its Consequences. Sinauer Assoc., Sunderland, MA.

Thompson, J. N., W. Wehling & R. Podolsky, 1990. Evolutionary genetics of host use in swallowtail butterflies. Nature. 344: 148-150.

Tsacas, L. & G. Bächli, 1981. *Drosophila sechellia*, n. sp., huitième espèce du sousgroupe *melanogaster* des iles Séchelles [Diptera, Drosophilidae]. Rev. Fr. Entom. (NS), 3: 146-150.

Wood, T. K. & M. C. Keese, 1990. Host-plant assortative mating in *Enchenopa* treehoppers. Evolution. 44: 619-628.

Wood, T. K. & K. L. Olmstead & S. I. Guttman, 1990. Insect phenology mediated by host-plant water relations. Evolution. 44: 629-636.