Journal of Insect Behavior, Vol. 3, No. 5, 1990

Effect of Adult Experience on Oviposition Choice and Short-Distance Attraction in *Drosophila buzzatii*

Philip W. Hedrick,[1,3] J. S. F. Barker,[2] and T. Armstrong[2]

Accepted February 14, 1990; revised April 12, 1990

In a series of experiments, no consistent effect of adult experience, i.e., exposure to the naturally occurring yeasts, Candida sonorensis *and* Clavispora opuntiae, *on oviposition choice or short-distance attraction in inbred lines of* Drosophila buzzatii *was found. The lack of consistent effect on oviposition choice was also found in one experiment in which the flies were starved and in another experiment in which choice was determined on 2 consecutive days.*

KEY WORDS: experience; oviposition preference; *Drosophila buzzatii*; yeasts; genetic variation.

INTRODUCTION

An often-cited explanation for the high amount of genetic variation in natural populations is that this variation confers adaptation to variation in aspects of the natural environment, such as temperature, food, and humidity (see discussion by Hedrick, 1986). When given genotypes choose habitats or niches for oviposition in which they have a high fitness, maintenance of genetic polymorphism is even more likely (e.g., Templeton and Rothman, 1981; Hedrick, 1990a, b). Furthermore, when previous experience enhances the choice of favored resources by given genotypes, then the conditions for polymorphism under some conditions are very robust (Jaenike, 1988).

There are a number of experiments suggesting that previous experience influences resource use in insects (see references cited by Papaj and Prokopy,

[1] Department of Biology, Pennsylvania State University, University Park, Pennsylvania 16802.
[2] Department of Animal Science, University of New England, Armidale, New South Wales 2351, Australia.
[3] To whom correspondence should be addressed.

0892-7553/90/0900-0689$06.00/0

1989). In particular, early adult experience appears to influence oviposition choice on different foods by female *Drosophila* (e.g., Hoffmann, 1988; Jaenike, 1988). Given that there is a genetic basis for oviposition choice in *Drosophila* and that previous experience will enhance this choice, it is likely that genetic variation at loci affecting choice will be maintained over time and potentially influence linked loci influencing performance on these resources. However, it also appears that the experimental system may influence the effects of experience. Hoffmann (1985) found strong positive experience effects on oviposition in small petri dishes but no effects in a population cage. It is therefore important to test for the effect of experience in multiple experimental systems.

Yeasts occurring in the natural habitat of *D. buzzatii*, i.e., rotting cactus, provide a major food resource for this *Drosophila* species. Detailed studies of these yeasts have demonstrated that there is extensive heterogeneity in yeast species abundance—on a geographical scale (among localities), between seasons within a locality, on a microgeographical scale (between rots within a locality at the same time), and even within a rot, both spatially and temporally (Barker *et al.*, 1983, 1984, 1987). In addition, *D. buzzatii* has differential preferences for yeast species for feeding and oviposition and the potential exists for variation in yeast species abundance to contribute to the maintenance of genetic variation in *D. buzzatii* (Vacek *et al.*, 1985). There is also some evidence of genetically based habitat selection for oviposition on different yeasts in this species (Barker *et al.*, 1986; Barker, 1990).

In these previous experiments, the *D. buzzatii* tested were naive, i.e., the tested individuals had no previous exposure to the various yeast species. Because it is known that Diptera can learn the cues associated with given hosts (feeding and oviposition substrates) and, as a result, choose hosts preferentially, we set up experiments to examine whether previous experience is important in the choice of yeast species by *D. buzzatii*.

MATERIALS AND METHODS

The four different experiments reported here used the same general methodology. We describe these general procedures in experiment 1 and, under the descriptions of the other experiments, give any differences between them and experiment 1.

Experiment 1. The first experiment was designed to characterize differences in yeast choice in a number of inbred lines. We examined four replicates of six inbred lines of *D. buzzatii* from each of four natural populations from Australia: Masthead Island (151°43′E, 23°32′S), Hemmant (153°03′E, 27°27′S), Metz Gorge (151°53′E, 30°35′S), and O'Hara (150°39′E, 32°26′S). These lines had been maintained by brother–sister mating on an autoclaved sucrose–yeast–cactus medium (Starmer and Barker, 1986) seeded with live *Saccharomyces cere-*

visiae for 10 to 17 generations prior to the initiation of the experiment. In preparation for this experiment, adults from each line were stored 10 pairs/vial in 75 × 25-mm vials for 24 h then transferred to 150-ml bottles, and discarded 2 days later. Progeny emerging in the bottles were collected daily, stored at 10 pairs/vial in vials with live *S. cerevisiae*, and transferred to fresh vials every 2 days until used in the experiment.

The two yeast species used in the choice test, *Candida sonorensis* and *Clavispora opuntiae*, were chosen from those known to be most abundant in cactus rots in Australia (Barker *et al.*, 1984) and among those most preferred for oviposition by *D. buzzatii* females (Vacek *et al.*, 1985). A bacterial community was used with each yeast species in order to simulate, at least partly, the microorganism composition of natural rots. This bacterial community comprised six strains (putatively different species, but the only two identified to species are *Xanthomonas* sp. and *Micrococcus kristinae*), which had been isolated from natural rots.

Cactus homogenate was used in yeast and bacterial community inoculum preparation and for experimental medium. Field-collected *Opuntia stricta* cladodes were cut into 3-cm squares, covered with water, autoclaved for 40 min, and thoroughly blended. All homogenates were pooled and mixed well to make one batch which was again autoclaved and stored until needed.

Each yeast species, together with the six bacteria strains, was presented to the flies growing on small disks of medium. Yeasts were grown for 48 h on complete medium agar plates. Standard yeast suspensions then were prepared by the nonphotometric method of Van der Walt (1970). Each bacterium strain was cultured in a 50% cactus homogenate liquid medium. Equal volumes of these bacteria cultures were mixed, and 1.5 ml of this mixture was added to 6 ml of each standardized yeast suspension. Each mixed bacteria/yeast inoculum was spread at 1 ml/200 cm^2 on to 3 to 4-mm-thick slabs of autoclaved cactus homogenate in 15-cm petri dishes and incubated at 25°C for 48 h, at which time a thick lawn of approximate plateau-phase growth was reached. The cactus homogenate slabs were 10% *O. stricta* homogenate and 1.5% agar.

On the morning that oviposition preference tests were set up, disks 1.2 cm in diameter were cut from each of the 48-h microbial culture slabs and placed in the oviposition test chambers. These chambers were 15-cm petri dishes with a cotton stoppered hole in the lid (for addition of flies) and a filter paper in the base moistened with sterile water. Twelve disks (six of each yeast species) were placed alternately on the filter paper around the periphery. Also on the morning that tests were set up, when the experimental flies were 6 days old, the stored flies of each line were pooled, and four lots of 15 females taken under light CO_2 anesthesia and put in vials with 2% agar. From 2:00 to 2:30 PM, the females were added to the oviposition preference chambers, and the chambers arranged randomly by strain and treatment and placed in a dark incubator at

25°C and 75% relative humidity. The females were removed the next morning at 9 AM, and the eggs on each disk counted.

Experiment 2. Four of the twenty-four inbred lines were chosen from experiment 1 for experiments 2–4 (see Results). In these experiments, flies were produced and provided experience as follows. Parents for each line were set up in vials and allowed to lay eggs for 24–48 h. Six days after setting up the vials, plastic sleeves (with an outside diameter equal to the inside diameter of vials) were inserted into each vial as pupation sites. Five days later, the sleeves were removed and attached pupae sterilized (yeast and bacteria on the external surface were killed) by immersion in Chlorox bleach solution (4%) for 10 min, transferred twice through 0.7% NaCl solution (5 min each), and rinsed in sterile water. Each sleeve was then inserted into a vial with the bacterial community only, or bacteria and *C. sonorensis*, or bacteria and *Cl. opuntiae*. Emerging adults thus were exposed immediately to the control (bacteria only) or to a yeast treatment. After 2 days, these adults were collected and stored on a fresh appropriate treatment vial (10 pairs/vial). They were again transferred to fresh treatment vials 2 days later. Treatment vials were prepared 48 h before use by adding three drops of mixed bacteria/yeast suspension (prepared as in experiment 1) to sucrose–yeast–cactus medium (diluted with 1 vol water to 2 vol medium) and incubating at 25°C.

For each inbred line in experiment 2, 15 six- or seven-day-old females from each of the three experience treatments were placed singly in 9-cm petri dishes with four disks (two of each yeast species). In this experiment, the bacteria and yeasts grew only sparsely on the diluted cactus medium in the treatment vials, and as a result, the flies were in a starved condition at the time they were given the choice between the yeasts.

Experiment 3. The format was identical to that of experiment 2, with two exceptions. First, the bacteria and yeasts in the treatment vials were grown on a medium (8 g agar, 36 g sucrose, 200 g cactus homogenate, 5 g yeast extract, 22.5 g tryptone, 7.5 g soya peptone, and 1270 ml water) that produced a luxuriant growth in 48 h after seeding, i.e., when adults commenced emergence into the vials, or when flies were transferred to fresh vials. Second, when females were removed from the oviposition test chambers the morning after initiation, they were stored in 2% agar vials until a repeat choice test was set up from 2:00 PM that afternoon.

Experiment 4. The flies in this experiment were given experience in the same manner as in experiment 3. However, the experiment was designed to test the short-distance attraction of the two yeast species using a Y-tube olfactometer. This apparatus (modified from Fuyama, 1976) consists of four Pyrex cylinders: a start cylinder for initial holding of flies, a choice cylinder, and two trap cylinders. The start and choice cylinders are separated by a sliding shutter. Once a fly moves from the choice cylinder to a trap cylinder through a small

funnel, it cannot return. Treatment vials (same as conditioning vials) were attached to the trap cylinders (one with *C. sonorensis*, the other with *Cl. opuntiae*) 15–30 min before flies were added. Odor passes through the Y-tube by simple diffusion. The responses were evaluated by an olfactory index calculated as N_1/N, where N_1 is the number of flies on yeast 1 (*C. sonorensis*) and N is the total number of flies on both yeasts.

When flies were 7 (some 6) days old, males and females were separated, dusted with microfluorescent pigment (Crumpacker, 1974; Moth and Barker, 1975) according to treatment, and stored in 2% agar vials for 4 h. At 1:45 PM flies were introduced to the start cylinders of the olfactometers: 45 flies (15 from each conditioning treatment) of one sex from one line in any one Y-tube. Each line–sex combination was replicated three times, making a total of 24 Y-tubes. At 2:00 PM, the Y-tubes were placed in a dark incubator at 25°C and the shutters opened. The following morning (9:00 AM), flies were removed from the trap cylinders, etherized, and counted under a fluorescent lamp.

RESULTS

The mean proportion of eggs (over replicates) laid on *C. sonorensis* for each of the 24 inbred lines in experiment 1 showed substantial variation from a low proportion of 0.376 to a high of 0.728 (Table I). ANOVA showed a significant effect for line within locality ($P < 0.05$), while the locality effect was not significant. Using these results, one line with a high proportion (O'Hara 5), one line with a low proportion (Masthead 3), and two intermediate lines (Masthead 4 and O'Hara 6) were chosen for the other three experiments.

In experiment 2 for each line × treatment combination, 15 females were individually tested, a proportion of which laid no or very few eggs in the test period. The number of females for each combination that laid one or more eggs

Table I. Mean Proportions of Eggs in Experiment 1 Laid on Media Inoculated with *C. sonorensis*, for 24 Inbred Lines (6 from Each of 4 Locations)[a]

	Locality			
Line	Hemmant	Masthead	Metz	O'Hara
1	0.504	0.719	0.592	0.518
2	0.562	0.546	0.529	0.581
3	0.540	0.450[a]	0.611	0.572
4	0.673	0.611[a]	0.505	0.569
5	0.523	0.677	0.543	0.728[b]
6	0.430	0.636	0.376	0.609[b]
Average	0.539	0.606	0.526	0.596

[a]The overall mean is 0.567.
[b]Indicates the four lines used in the later experiments.

ranged from 9 to 15 (average, 13.4). The mean proportions of eggs on *C. sonorensis* for these females show no consistent experience effect (Table II). For two of the lines (Masthead 4 and O'Hara 5), the proportions are higher for females experienced with *C. sonorensis* than for those experienced with *Cl. opuntiae* (as expected for a positive effect of experience), but the proportions are reversed for the other two lines. Analysis of variance showed no significant effects for lines, treatments, or the interaction of lines and treatments. As females laying very few eggs might not be expressing true preferences (because of prior starvation), the mean proportions were recalculated after discarding results for females laying less than 10 eggs. These means were for an average of 11.4 females for each line × treatment combination, but the ANOVA effects were unchanged.

The mean proportions of eggs on *C. sonorensis* in experiment 3 again show no consistent effects of experience (Table III). One line (Masthead 4) had a higher proportion for females experienced with *C. sonorensis* on Day 1, while two other lines (Masthead 3 and O'Hara 6) show this result for Day 2. However, analysis of variance of the proportions showed no significant effects, for either Day 1 or Day 2. Regression analysis of the proportions on Day 2 on those on Day 1, gave homogeneous slopes for the line × treatment combinations, a pooled regression coefficient of 0.189 ($P = 0.069$), and a pooled correlation coefficient of 0.406. As in experiment 2, all results were similar for analyses done after discarding results for females laying fewer than 10 eggs.

Significantly fewer eggs were laid by females that had been provided bacteria only on both Day 1 and Day 2. Effects of starvation on the experiment 2 females are apparent in numbers of eggs laid–an average of 22.3 eggs/female for experiment 2, as compared with 40.4 and 31.9 for experiment 3 females on days 1 and 2, respectively.

The mean proportions of flies that chose *C. sonorensis* in the Y-tube (experiment 4) again show no consistent effects of experience (Table IV) and analysis of variance showed no significant effects. The average numbers of flies that

Table II. Mean Proportions (Standard Errors) of Eggs in Experiment 2 Laid on *C. sonorensis* for Individually Tested "Starved" Females

Experience treatment	Locality and line number				
	Masthead		O'Hara		
	3	4	5	6	Average
Bacteria only	0.423 (0.143)	0.688 (0.083)	0.375 (0.104)	0.647 (0.148)	0.533 (0.060)
C. sonorensis	0.481 (0.128)	0.558 (0.121)	0.580 (0.124)	0.395 (0.132)	0.509 (0.062)
Cl. opuntiae	0.500 (0.139)	0.540 (0.115)	0.476 (0.123)	0.571 (0.137)	0.523 (0.063)
Average	0.471 (0.077)	0.596 (0.062)	0.479 (0.068)	0.530 (0.080)	0.521 (0.036)

Table III. Mean Proportions (Standard Errors) of Eggs in Experiment 3 Laid on *C. sonorensis*

Experience treatment	Locality and line number				
	Masthead		O'Hara		
	3	4	5	6	Average
Day 1					
Bacteria only	0.782 (0.122)	0.759 (0.115)	0.596 (0.089)	0.612 (0.108)	0.667 (0.055)
C. sonorensis	0.524 (0.107)	0.856 (0.056)	0.572 (0.079)	0.631 (0.080)	0.633 (0.045)
Cl. opuntiae	0.588 (0.086)	0.698 (0.111)	0.606 (0.076)	0.692 (0.095)	0.642 (0.045)
Average	0.620 (0.061)	0.780 (0.052)	0.591 (0.046)	0.646 (0.053)	0.646 (0.027)
Day 2					
Bacteria only	0.800 (0.133)	0.399 (0.127)	0.670 (0.091)	0.796 (0.100)	0.686 (0.059)
C. sonorensis	0.564 (0.125)	0.647 (0.068)	0.562 (0.099)	0.714 (0.113)	0.618 (0.051)
Cl. opuntiae	0.406 (0.112)	0.744 (0.057)	0.627 (0.069)	0.433 (0.095)	0.544 (0.047)
Average	0.564 (0.074)	0.623 (0.050)	0.618 (0.049)	0.636 (0.063)	0.609 (0.030)

Table IV. Mean Proportions (Standard Errors) of Flies in Experiment 4 that Choose *C. sonorensis* When Given the Choice of Two Yeasts in a Y-Tube Olfactometer

Experience treatment	Sex	Locality and line number				
		Masthead		O'Hara		
		3	4	5	6	Average
Bacteria only	M	0.464 (0.146)	0.573 (0.113)	0.700 (0.300)	0.644 (0.178)	0.595 (0.088)
	F	0.411 (0.106)	0.411 (0.070)	0.758 (0.093)	0.444 (0.099)	0.506 (0.059)
C. sonorensis	M	0.535 (0.137)	0.352 (0.068)	0.662 (0.180)	0.466 (0.126)	0.504 (0.066)
	F	0.578 (0.118)	0.444 (0.118)	0.674 (0.007)	0.503 (0.091)	0.550 (0.048)
Cl. opuntiae	M	0.515 (0.107)	0.581 (0.171)	0.500 (0.165)	0.603 (0.032)	0.550 (0.059)
	F	0.373 (0.029)	0.582 (0.138)	0.476 (0.133)	0.510 (0.127)	0.485 (0.054)
Average		0.480 (0.043)	0.491 (0.046)	0.628 (0.064)	0.529 (0.044)	0.532 (0.025)

responded (i.e., moved from the start tube to one or other choice tube) were 12.4 for males and 14.1 for females.

DISCUSSION

In the past decade, substantial research has focused on the behavior, ecology, and evolution of the cactophilic *Drosophila* (for reviews see Barker and Starmer, 1982; Barker *et al.*, 1990). With this background and specific data demonstrating oviposition choice in *D. buzzatii*, this system is obviously an appropriate one for examining the influence of experience on oviposition choice

and short-distance attraction. In our experiments, we examined the influence of early adult experience with different yeasts on these traits. However, we were not able to demonstrate any consistent influence of experience on either behavior.

Because we initially felt, based on the work of others, that experience could play an important role in these behavioral traits, these results are somewhat surprising. There are several possible explanations for these results. First, the flies were not able to differentiate between these yeasts. This possibility we feel we can reject because of the significant among-line effect shown in experiment 1 and previous studies demonstrating strong differential choice among yeasts (e.g., Vacek *et al.*, 1985; Barker, 1990). Second, the test system for some reason did not detect the effect of experience. For example, if the effect of experience is lost after the first few eggs are laid, then this influence may be diluted by further random choice. However, as there was no positive effect of experience for initial attraction in the Y-tube experiment, such a possibility is unlikely. One limitation of these experiments is that we, not the flies, determined the length of the experience, a factor that may influence later behavior (Hoffman, 1988). Third, in nature the areas in which different yeasts are found may differ in other attributes, such as the bacterial flora. These other characteristics may be the relevant cues involved in learning rather than the yeasts themselves. Fourth, *D. buzzatii* oviposition and short-distance attraction behavior may not be influenced by previous experience with yeasts because these flies do not have the ability to choose yeasts based on past experience.

One other feature of the results warrants comment and further study. With only four inbred lines tested, the data are limited, but the ranking of lines for proportion of eggs laid on *C. sonorensis* varies between experiments. This is particularly marked for O'Hara 5, chosen from experiment 1 because of its high preference. Thus oviposition choice may be affected by experimental conditions: experiment 1 flies were maintained on *S. cerevisiae* before testing, as compared with the test yeasts in experiments 2 and 3, while flies in the latter two experiments differed in being starved or well fed prior to testing.

The effect of past experience on behavior is not universal and it may not be demonstrable even in a well-defined and understood system. Obviously then, experience may not be a factor that will relax the conditions for a balanced polymorphism in all species.

ACKNOWLEDGMENTS

Phil Hedrick thanks the University of New England for the award of a Visiting Research Fellowship which enabled him to participate in this research. We all thank Ary Hoffmann and Tom Starmer, who made a number of helpful comments on the manuscript, and are indebted to Jim Fogleman for identification of the bacteria strains. Excellent laboratory support was provided by

Annette Edmonds, Chris Leger, and Don Fredline. The work was supported by a grant to J.S.F.B. from the Australian Research Grants Scheme.

REFERENCES

Barker, J. S. F. (1990). Experimental analysis of habitat selection and maintenance of genetic variation. In Barker, J. S. F., Starmer, W. T., and MacIntyre, R. J. (eds.), *Ecological and Evolutionary Genetics of Drosophila*, Plenum Press, New York, pp. 161–175.

Barker, J. S. F., and Starmer, W. T. (eds.) (1982). *Ecological Genetics and Evolution. The Cactus-Yeast-Drosophila Model System*, Academic, New York.

Barker, J. S. F., Toll, G. L., East, P. D., Miranda, M., and Phaff, H. J. (1983). Heterogeneity of the yeast flora in the breeding sites of cactophilic *Drosophila*. *Can. J. Microbiol.* **29:** 6–14.

Barker, J. S. F., East, P. D., Phaff, H. J., and Miranda, M. (1984). The ecology of the yeast flora in necrotic *Opuntia* cacti and of associated *Drosophila* in Australia. *Microb. Ecol.* **10:** 379–399.

Barker, J. S. F., Vacek, D. C., East, P. D., and Starmer, W. T. (1986). Allozyme genotypes of *Drosophila buzzatii*: Feeding and oviposition preferences for microbial species, and habitat selection. *Aust. J. Biol. Sci.* **39:** 47–58.

Barker, J. S. F., Starmer, W. T., and Vacek, D. C. (1987). Analysis of spatial and temporal variation in the community structure of yeasts associated with decaying *Opuntia* cactus. *Microb. Ecol.* **14:** *267–276.*

Barker, J. S. F., Starmer, W. T., and MacIntyre, R. J. (1990). *Ecological and Evolutionary Genetics of Drosophila*, Plenum Press, New York.

Crumpacker, D. W. (1974). The use of micronized fluorescent dusts to mark adult *Drosophila pseudoobscura*. *Am. Midl. Nat.* **91:** 118–129.

Fuyama, Y. (1976). Behavior genetics of olfactory responses in *Drosophila*. I. Olfactometry and strain differences in *Drosophila melanogaster*. *Behav. Genet.* **6:** 407–420.

Hedrick, P. W. (1986). Genetic polymorphism in heterogeneous environments: A decade later. *Annu. Rev. Ecol. Syst.* **17:** 535–566.

Hedrick, P. W. (1990a). Theoretical analysis of habitat selection and the maintenance of genetic variation. In Barker, J. S. F., Starmer, W. T., and MacIntyre, R. J. (eds.), *Ecological and Evolutionary Genetics of Drosophila*, Plenum Press, New York, pp. 209–227.

Hedrick, P. W. (1990b). Genotypic-specific habitat selection: A new model and its application. *Heredity* (in press).

Hoffmann, A. A. (1985). Effects of experience on oviposition and attraction in *Drosophila*: Comparing apples and oranges. *Am. Nat.* **126:** 41–51.

Hoffmann, A. A. (1988). Early adult experience in *Drosophila melanogaster*. *J. Insect Physiol.* **34:** 197–204.

Jaenike, J. (1988). Effects of early adult experience on host selection in insects: Some experimental and theoretical results. *J. Insect Behav.* **1:** 3–15.

Moth, J. J., and Barker, J. S. F. (1975). Micronized fluorescent dusts for marking *Drosophila* adults. *J. Nat. Hist.* **9:** 393–396.

Papaj, D. R., and Prokopy, R. J. (1989). Ecological and evolutionary aspects of learning in phytophagous insects. *Annu. Rev. Entomol.* **34:** 315–350.

Starmer, W. T., and Barker, J. S. F. (1986). Ecological genetics of the *Adh-1* locus of *Drosophila buzzatii*. *Biol. J. Linn. Soc.* **28:** 373–385.

Templeton, A. R., and Rothman, L. D. (1981). Evolution in fine-grained environments. II. Habitat selection as a homeostatic mechanism. *Theor. Pop. Biol.* **19:** 326–340.

Vacek, D. C., East, P. D., Barker, J. S. F., and Soliman, M. H. (1985). Feeding and oviposition preferences of *Drosophila buzzatii* for microbial species isolated from its natural environment. *Biol. J. Linn. Soc.* **24:** 175–187.

Van der Walt, J. P. (1970). Criteria and methods used in classification. In Lodder, J. (ed.), *The Yeasts: A Taxonomic Study*, North-Holland, Amsterdam, pp. 34–113.