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GENETIC VARIATION IN CACTOPHILIC *DROSOPHILA* FOR OVIPOSITION ON NATURAL YEAST SUBSTRATES

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Abstract.—Theory predicts that environmental heterogeneity in space or in time can maintain genetic polymorphism. Stable polymorphisms are expected to be more readily maintained if there are genotype specific habitat preferences. Genotype specific preferences for oviposition sites in *Drosophila* could be a major factor promoting habitat selection, and thus the maintenance of genetic variation. This hypothesis is being tested using the cactophilic species, *D. buzzatii* and *D. aldrichi,* where available evidence indicates a potential for such habitat selection, the habitats (oviposition sites) being yeast species found in the natural environment of these flies (cactus rots). Genetic variation for oviposition preferences was tested using isofemale lines—for *D. buzzatii,* a total of 60 lines from seven localities widely distributed through the species range in Australia, and for *D. aldrichi,* 21 lines from three of these localities. Females were given a choice of five yeast species as oviposition sites. Genetic variation for oviposition preferences on these natural substrates was demonstrated. There was significant variation among isofemale lines within populations in their patterns of preferences for oviposition on the five yeast species. However, analyses of preferences for each yeast species separately showed that the genetic variation for preferences relates to only three of the five species. Heritabilities of individual female preferences for these three species were low, ranging up to 9%. Little geographic differentiation was apparent among populations, most likely due to similar selection regimes within each population. Within populations, this kind of habitat selection could act to maintain polymorphisms, both at loci determining the habitat preferences and at other loci in linkage disequilibrium with them.

Key words.—Cactophilic *Drosophila, Drosophila*-yeast interactions, habitat selection, oviposition site preference.

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The maintenance of extensive genetic variation in natural populations (Brown, 1979; Nevo et al., 1984) remains as a major unsolved problem in evolutionary genetics. Theory shows that environmental heterogeneity in space or in time can maintain genetic polymorphisms (Karlin, 1982; Hedrick, 1986), although the conditions may be stringent for some models of natural selection (Maynard Smith and Hoekstra, 1980; Hoekstra et al., 1985). However, stable polymorphisms are more readily maintained if there is genotype specific habitat selection, with differential selection in the different habitats (Maynard Smith, 1970; Taylor, 1976; Templeton and Rothman, 1981; Garcia-Dorado, 1986, 1987; Hedrick, 1990*a,* 1990*b*). Other models developed by Rausher (1984) and Diehl and Bush (1989) show that genetic variation can be maintained at a locus affecting habitat preference, even without this locus affecting fitness in the different habitats. There is little empirical evidence for genotype specific habitat selection, and none demonstrating such selection acting to maintain genetic polymorphisms in natural populations.

To be definitive, studies of resource use need to: (i) distinguish genetic variation for habitat selection from effects of habituation or experience (Jaenike, 1983; Hoffmann, 1985) or of the physiological state of the organism (Hoffmann and Turelli, 1985); (ii) use habitats that are relevant to natural populations; and (iii) measure traits involved in the use of the resource, e.g., for feeding or oviposition. There is some evidence for genetic variation in resource use (food or microhabitat) within populations of insects (Futuyma and Peterson, 1985), in oviposition host preference in the polyphagous *Drosophila tripunctata* (Jaenike and Grimaldi, 1983; Jaenike, 1989) and in oviposition preferences for different laboratory media in *D. melanogaster* (Bird and Semeonoff, 1986), and genetic variation in olfactory response affects habitats selected in *D. melanogaster* (Hoffmann et al., 1984). For *Drosophila,* response to different oviposition sites may be most important in de-

termining niche separation of species (Carson, 1971; Shorrocks, 1975). Extending this to within species, genotype specific preferences for oviposition sites could be a major factor promoting habitat selection. This hypothesis is the basis for the studies reported here.

The cactophilic *Drosophila,* because of their relatively well-known ecology (Barker and Starmer, 1982), provide a model system for analysis of habitat selection (Barker, 1990). Two species, *D. buzzatii* and *D. aldrichi* (both in the mulleri subgroup of the repleta group), have colonized Australia in association with various species of *Opuntia* cactus (Sokal et al., 1987). The niche of these flies is necrotic tissue (rots) of cactus cladodes. Females oviposit in the rots, and larvae and adults feed on rot microorganisms (yeasts and bacteria). Volatiles produced by these microorganisms most likely provide cues to flies seeking feeding or oviposition sites (Fogleman, 1982; Fogleman and Abril, 1990).

Cactus rots are spatially and temporally heterogeneous in abundance and frequency of yeasts (Barker et al., 1983, 1988), and this heterogeneity in the environment of the flies could provide a basis for habitat selection. For *D. aldrichi,* little information is available, but adults have been shown to be attracted differentially to yeast species in both laboratory and field experiments (Barker et al., 1981*a,* 1981*b*). For *D. buzzatii,* available evidence indicates a potential for such habitat selection, in that adults feed differentially on the yeast species found in natural cactus rots, females prefer as oviposition sites those yeasts that in general are best for larval development (Vacek et al., 1985), and there is indirect evidence for genetic variation in oviposition preferences on different yeasts (Barker et al., 1986). On the other hand, no consistent effect of adult experience on oviposition preferences for yeast species has been found for *D. buzzatii* (Hedrick et al., 1990).

Because olfactory responses to species of yeast and other microorganisms are likely to provide the primary cue to females seeking an oviposition site, their responses in natural populations can occur at two levels, viz. between rots and within rots. Natural rots contain 1–5 yeast species with an average of about 2–3 (Barker et al., 1983, 1984), so that choice between rots likely depends on long distance attraction and responses to combinations of yeast species, bacteria species and other factors such as age of the rot.

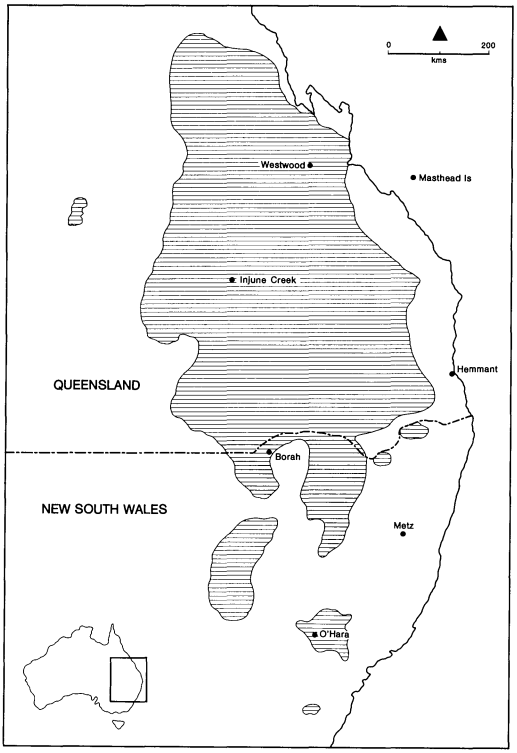

FIG. 1. Locations of the populations from which isofemale lines were collected. The hatched areas include the distribution of the main *Opuntia* infestations in 1920.

The present experiments are designed to assay female behavior at the second level—after they have found a rot, i.e., choice within rots where the yeast species distribution is patchy (Barker et al., 1988). Females were given a simultaneous choice of five cactophilic yeast species as oviposition sites and female preferences were measured by the number of eggs laid on each yeast.

MATERIALS AND METHODS

Experiment 1 used eight isofemale lines of *D. buzzatii* from each of four populations (Fig. 1): 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 in the laboratory on an auto-

claved sucrose-yeast-cactus medium (Starmer and Barker, 1986) seeded with live *Saccharomyces cerevisiae* for 20, 13, 10, and 12 generations respectively prior to initiation of the experiment. Experiment 2 used seven isofemale lines of both *D. buzzatii* and *D. aldrichi* from each of three populations: Westwood (150°08′E, 23°38′S), Injune Creek (148°33′E, 25°52′S), and Borah (149°24′E, 28°55′S). These lines had been maintained in the laboratory in the same way as those above, but for only four generations prior to the experiment. Experiment 3 used the same seven isofemale lines of *D. aldrichi* from each of Westwood, Injune Creek, and Borah (except for the replacement of one line from Borah). At this time, they had been maintained in the laboratory for nine generations. At the same time, seven newly collected isofemale lines of *D. buzzatii* from Metz Gorge (one generation in the laboratory) were tested. Experiment 4 retested the *D. buzzatii* isofemale lines of Experiments 2 and 3. At initiation of this experiment, these lines had been maintained in the laboratory for 17 generations (Experiment 2 lines) and for eight generations (Experiment 3 lines). During laboratory maintenance, none of the lines used in any experiment were exposed to cactophilic yeasts or bacteria.

Except for some details for *D. aldrichi* in Experiment 3 (noted below), all procedures were identical for all experiments. The autoclaved sucrose-yeast-cactus medium was used for all adult storage and breeding of experimental flies. For each line, adults from stock cultures were stored 10 pairs/vial in 75 mm × 25 mm vials for 24 hours, then transferred to 150 ml bottles, and discarded two 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 two days until used in an experiment.

The five yeast species used, viz. *Candida sonorensis* (Cs), *Pichia cactophila* (Pc), *Clavispora opuntiae* (*Clo*), *Candida mucilagina* (*Cm*), *and Cryptococcus cereanus* (*Crc*), were chosen from those known to be most abundant in cactus rots in Australia (Barker et al., 1984), and to provide a wide range of expected preferences for oviposition by *D. buzzatii* females (Vacek et al., 1985). A bacterial community was grown with each yeast species, in order to simulate the microorganism composition of natural rots. This comprised six strains isolated from necrotic *Opuntia* tissue: *Erwinia cacticida* (Alcorn et al., 1991), *Micrococcus kristinae, Xanthomonas* sp., and three as yet unidentified but different species. One batch of cactus homogenate was used in all yeast and bacterial community inoculum preparation, and all experimental media. 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 at −15°C until needed.

Each yeast species, mixed with the six bacteria strains, was presented to the flies growing on small discs of medium. Yeasts were passed through a 48 hr growth period on complete medium (yeast extract-malt extract-agar). Standard yeast suspensions then were prepared by the nonphotometric method of Van der Walt (1970). Each bacterial strain was cultured in a 50% cactus homogenate liquid medium. Equal volumes of these bacteria cultures were mixed, and 1.5 ml of this mix added to 6 ml of each standardized yeast suspension. Each mixed bacteria/yeast inoculum was spread at 1 ml/200 cm^2 on to 3–4 mm thick slabs of autoclaved homogenate in 15 cm petri dishes and incubated at 25°C for 48 hr, at which time a thick lawn of approximate plateau phase growth was reached. For Experiments 1 and 2 and for *D. buzzatii* in Experiments 3 and 4, the homogenate slabs were 10% *O. stricta* homogenate, 1.5% agar. For *D. aldrichi* in Experiment 3, the slabs were prepared as 67.5% *O. stricta* homogenate, 10% *S. cerevisiae* and 1.0% agar.

During the morning of the day that oviposition preference tests were begun in each experiment, the oviposition chambers and experimental females were prepared.

The oviposition chambers were 15 cm petri dishes with a cotton stoppered hole in the lid (for addition of flies) and a 14.3 cm diameter filter paper in the base moistened with 2.5 ml sterile water. Discs 1.2 cm in diameter were cut from each of the 48 hr microbial culture slabs. For each chamber,

fifteen discs (three of each yeast species) were placed on the filter paper around the periphery in a predefined order, such that any disc of a particular yeast was located between two of the other species [see Jaenike and Grimaldi (1983) for a similar procedure]. For all chambers of any one replicate, the same sequence of discs was used, but different sequences were used for each of the four replicates.

Twenty females were placed in each oviposition chamber, and these were prepared by pooling the stored flies of each line, separating four lots of 20 females under light CO_2 anaesthesia, and placing them in vials with a "Kimwipe" tissue pressed to the base and moistened with 1 ml distilled water. Between 2:15 and 3:15 p.m., the females were added to the oviposition preference chambers, and the chambers arranged in random order and placed in an incubator by 3:30 p.m. At 8:00 p.m. the following day, the chambers were moved from the incubator to a cold room at 0°C to prevent further egg development. The eggs on each disc were counted over the next few days. For Experiments 1, 2, and 4 and *D. buzzatii* in Experiment 3, the chambers were put in a dark incubator at 25°C, 75% R.H. Under the conditions of Experiment 2, *D. aldrichi* fecundity was poor, with no eggs laid in 6 of the 84 test chambers, and fewer than 20 eggs in eight additional chambers. The data set thus was reduced to four lines in each of the three populations, but even so, average numbers of eggs per chamber were much lower than for *D. buzzatii* (348 versus 683), and many discs had no eggs. For *D. aldrichi* in Experiment 3, the incubator was set on a 12 hr light, 12 hr dark cycle (0600–1800 hours light) at 28°C, 75% R.H.

The experimental females averaged 9 days old (range 5–11) in Experiment 1, 10 days (range 9–11) in Experiment 2, 9 days (range 8–10) in Experiment 3, and 11 days (range 10–11) in Experiment 4.

These females had been kept in vials with live *S. cerevisiae* prior to the preference testing, and would be expected to introduce this species to the test chambers. Further, movement of flies during the test period would cause cross-contamination between discs. These contaminants were unlikely to competitively multiply on the heavy initial growth, but to the extent that they were able to do so, the effect would be to reduce the sensitivity of the test.

Eggs per disc were the primary data, and results were analysed using analysis of variance of eggs per disc and of the proportions of eggs laid on each of the yeasts in each oviposition chamber. In all experiments, both variables showed significant departures from normality, and across yeasts, the variance generally increased with the mean. Therefore, Box-Cox-Bartlett transformations (Sokal and Rohlf, 1981) were done to normalize distributions and homogenize variances. For analysis of the proportions of eggs on each yeast, results for the generally least preferred yeast (*Cr. cereanus*) were deleted to remove dependence among yeast proportions (since the five proportions sum to unity). The oviposition preferences measured were based on large sample sizes, with mean numbers of eggs per chamber of 663 for *D. buzzatii* and 685 for *D. aldrichi.*

Results

D. buzzatii: *Experiment 1*

Analysis of variance of the number of eggs per disc, with populations, yeasts and replicates as fixed effects, and isofemale lines and discs as random, showed significant effects for yeasts ($P < 0.001$), populations ($P < 0.05$) and isofemale lines within populations ($P < 0.001$). The latter indicate genetic differences in fecundity, but the main result is the significant interaction for yeasts × isofemale lines within populations ($P < 0.001$), i.e., differences among isofemale lines in the relative numbers of eggs laid on the five yeast species. Such differences may result from specific preferences for oviposition substrates, but the patterns of preferences may be dependent on fecundity. That is, all strains may have the same rank order for preference but differ in sensitivity to the yeasts (Jaenike and Grimaldi, 1983). In this case, strains that are more fecund might be either more sensitive [i.e., a higher proportion of eggs laid on the preferred yeast(s)] or less sensitive (i.e., a more even distribution of eggs).

Any such effects of fecundity were not apparent in this experiment. First, many strains did differ in rank order of preferences

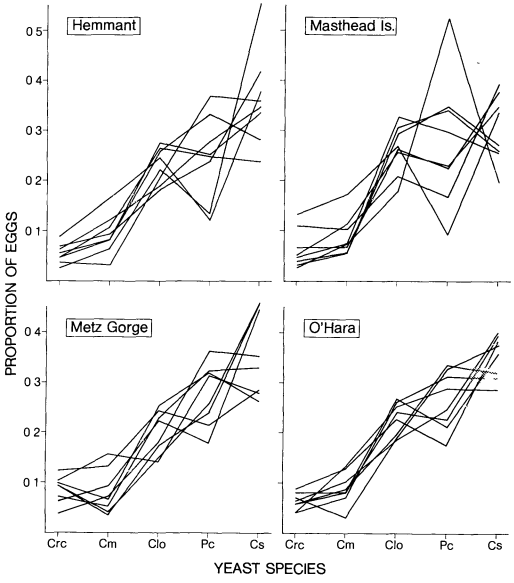

FIG. 2. *D. buzzatii*—Experiment 1: Proportions of eggs laid on each yeast species for each of eight isofemale lines from four populations. See Methods for abbreviations of yeast species.

(Fig. 2). Second, an index of sensitivity can be estimated as the variance of the proportions of eggs laid on each of the five yeasts. This index is a measure of deviation from random oviposition, i.e., no preference for any yeast or an expectation of 20% of the eggs laid on each yeast, so that a larger variance indicates greater sensitivity. Regression coefficients of this index on total egg number for each replicate were not significant, either overall or for each population (except O'Hara, Table 1).

Analysis of the proportions of eggs on each yeast essentially removes any effect of fecundity and specifically analyzes the pattern of oviposition on the five yeasts. For this analysis, populations, yeasts, and replicates were treated as fixed effects and isofemale lines as random (Table 2). The main effect for yeasts was highly significant ($P < 0.001$), as was the interaction for yeasts × isofemale lines within populations ($P < 0.001$). That is, there are differences among isofemale lines within populations in preferences for oviposition substrates.

D. buzzatii: *Experiment 2*

Analysis of variance of the number of eggs per disc gave similar results to those of Experiment 1, with significant effects for yeasts ($P < 0.001$), isofemale lines within populations ($P < 0.001$) and for yeasts × isofemale lines within populations ($P < 0.05$). In contrast to Experiment 1, regression coefficients of the sensitivity index (variance of the proportions of eggs laid on the five yeasts) on total egg number for each replicate (Table 1) were significant for the Borah and Injune Creek populations, and overall. However, the significant overall regression was determined primarily by eight points of

TABLE 1. Regression coefficients (b) for the variance of the proportions of eggs laid on the five yeasts on total egg number for each replicate.

		b × 10^5			b × 10^5
D. buzzatii Exp. 1	Overall	−0.884	*D. aldrichi* Exp. 2	Overall	−9.005**
	Hemmant	0.816		Borah	−12.260
	Masthead Is.	−1.716		Injune Ck	−4.751
	Metz Gorge	−0.983		Westwood	−10.040*
	O'Hara	−2.298*			
D. buzzatii Exp. 2	Overall	−2.252***	*D. aldrichi* Exp. 3	Overall	−0.874
	Borah	−3.980**		Borah	0.706
	Injune Ck	−1.878*		Injune Ck	−0.754
	Westwood	−0.547		Westwood	−1.852
D. buzzatii Exp. 3	Metz Gorge	−6.827*			
D. buzzatii Exp. 4	Overall	−1.353***			
	Borah	−0.307			
	Injune Ck	−0.385			
	Westwood	−1.537**			
	Metz Gorge	−2.750			

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 2. Analyses of variance of proportions of eggs per yeast (after Box-Cox-Bartlett transformation) for *D. buzzatii*.

Source of variation	Experiment 1			Experiment 2		
	df	Mean square	F†	*df*	Mean square	F†
Population (P)	3	0.00643	0.530 (1)	2	0.00113	0.176 (1)
Isolines/P (I/P)	28	0.01213 (1)	0.231 (3)	18	0.00643 (1)	0.160 (3)
Yeast (Y)	3	8.01989	78.530*** (2)	3	1.85636	25.658*** (2)
Y × P	9	0.05695	0.558 (2)	6	0.16565	2.290* (2)
Y × I/P	84	0.10213 (2)	1.947*** (3)	54	0.07235 (2)	1.800*** (3)
Residual	384	0.05247 (3)		252	0.04020 (3)	

Source of variation	Experiment 3			Experiment 4		
	df	Mean square	F†	*df*	Mean square	F†
Population (P)				3	0.08559	1.414 (1)
Isolines/P (I/P)	6	0.04618	0.330 (2)	24	0.06054	0.630 (3)
Yeast (Y)	3	2.23761	13.954*** (1)	3	1.16408	5.301** (2)
Y × P				9	0.17201	0.783 (2)
Y × I/P	18	0.16035 (1)	1.147 (2)	72	0.21960	2.285*** (3)
Residual	93	0.13986 (2)		336	0.09610	

† F-ratio with denominator mean square indicated in parentheses.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

high variance at low egg numbers, six of which were for Borah and two for Injune Creek.

Analysis of the proportions of eggs on each yeast (Table 2) gave highly significant effects ($P < 0.001$) for yeasts and for yeasts × isofemale lines within populations. In addition, the interaction for yeasts × populations was significant ($P < 0.05$).

As in Experiment 1, isofemale lines within populations differed in their rank order of yeast preferences, but the overall ranking of preferences (Fig. 3) was different from that in Experiment 1 (Fig. 4). Possible reasons for the difference were addressed in Experiment 4, and are discussed later.

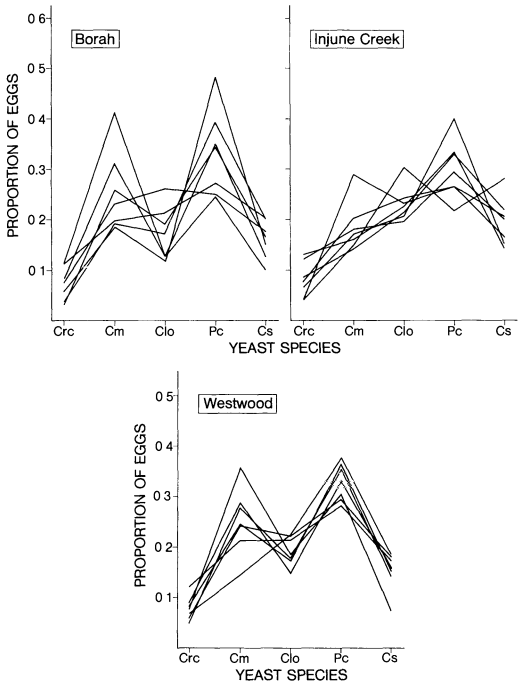

FIG. 3. *D. buzzattii*—Experiment 2: Proportions of eggs laid on each yeast species for each of seven isofemale lines from three populations.

D. buzzatii: *Experiment 3*

For the one population in this experiment, eggs per disc showed significant variation among isofemale lines and yeasts (as

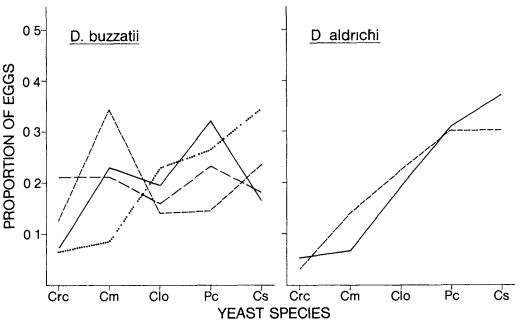

FIG. 4. Average proportions of eggs laid on each yeast species over all populations in each experiment. ····· Experiment 1, —— Experiment 2, – – – Experiment 3, — — — Experiment 4.

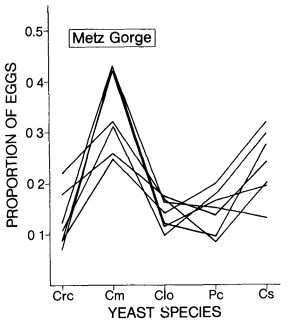

FIG. 5. *D. buzzattii*—Experiment 3: Proportions of eggs laid on each yeast species for each of seven isofemale lines from one population.

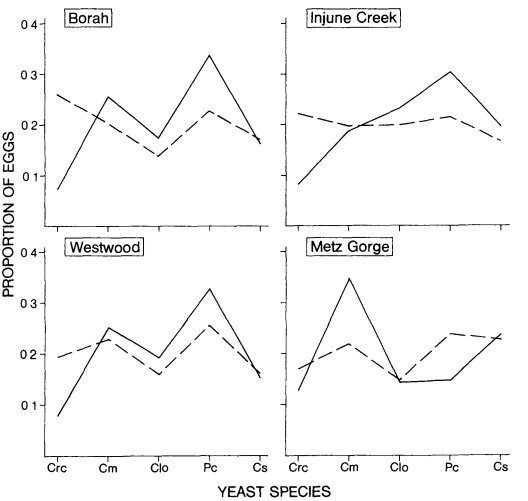

FIG. 6. *D. buzzatii*—Comparisons of the mean proportions of eggs laid on each yeast species for the same isofemale lines from Borah, Injune Creek, and Westwood (experiments 2 and 4), and from Metz Gorge (experiments 3 and 4). —— Experiments 2 and 3, – – – Experiment 4.

in Experiments 1 and 2), but the interaction of yeasts × isofemale lines was not significant. As for Experiment 2, the regression coefficient of the sensitivity index on total egg number for each replicate was significant (Table 1). Analysis of variance of the proportions of eggs on each yeast (Table 2) showed significant differences only among yeasts (Fig. 5).

D. buzzatii: *Experiment 4*

Analysis of variance of the number of eggs per disc gave similar results to those of previous experiments, with significant effects for yeasts ($P < 0.001$), isofemale lines within populations ($P < 0.001$), yeasts × isofemale lines within populations ($P < 0.001$), and for populations ($P < 0.05$). As in Experiment 2, there were significant regression coefficients of the sensitivity index on total egg number for each replicate, both overall and for the Westwood population (Table 1). Although the regression coefficient for the Metz population was not significant ($P = 0.085$), this population made a major contribution to the significant overall regression, as it had the highest mean variance and lowest total egg number.

Analysis of the proportions of eggs on each yeast (Table 2) again gave highly significant effects for yeasts ($P < 0.01$) and for yeasts × isofemale lines within populations ($P < 0.001$).

The isofemale lines in this experiment were the same as those in Experiment 2 (for Borah, Injune Creek, and Westwood) and Experiment 3 (for Metz), and the primary question was whether the yeast preferences expressed in these earlier experiments were repeatable after a further 13 or 7 generations of laboratory maintenance. The preference rankings of lines in the two experiments (Fig. 6) were not significantly different. For the three populations in Experiments 2 and 4, analysis of the combined data (including experiment as a fixed effect) gave a nonsignificant effect for experiment × yeast × isofemale lines within populations. The same was found for the combined analysis of Experiments 3 and 4. Thus these experiments provide no evidence for changes in oviposition preferences during laboratory maintenance.

D. aldrichi: *Experiment 2*

Because many discs had no eggs (see Methods), no transformation would normalize number of eggs per disc, and the basic datum was taken as eggs per yeast. Analysis of variance of the number of eggs per yeast (following Box-Cox-Bartlett transformation) gave significant effects only for isofemale lines within populations ($P < 0.001$) and for yeasts ($P < 0.001$). Analysis of the proportions of eggs on each yeast (Table 3, Fig. 7) gave a significant effect only for yeasts ($P < 0.001$).

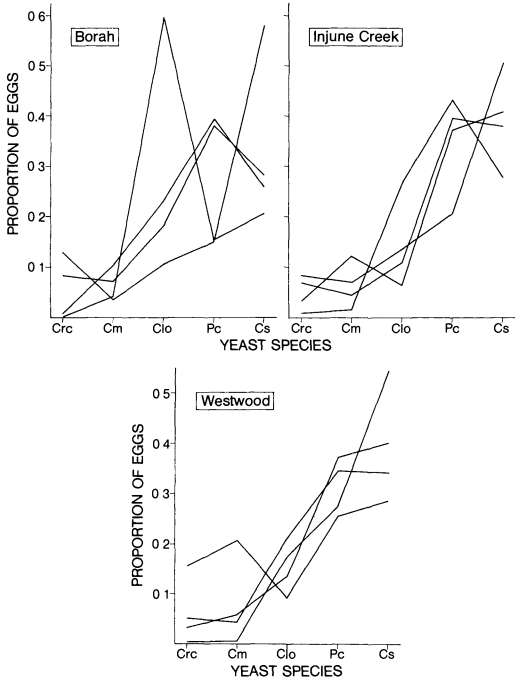

FIG. 7. *D. aldrichi*—Experiment 2: Proportions of eggs laid on each yeast species for each of four isofemale lines from three populations.

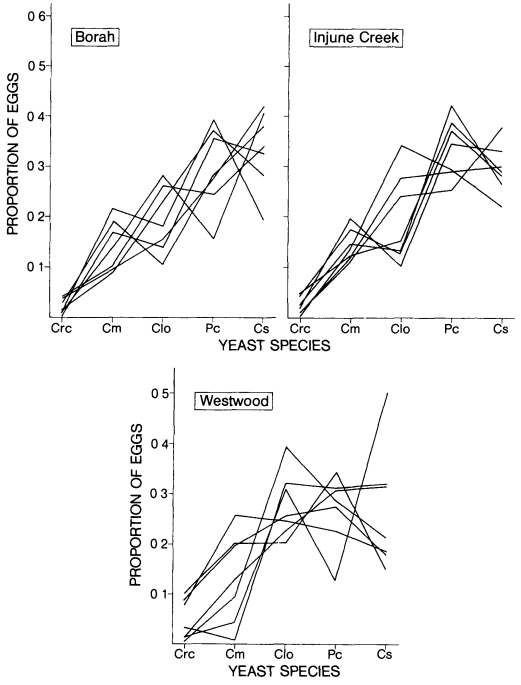

FIG. 8. *D. aldrichi*—Experiment 3: Proportions of eggs laid on each yeast species for each of seven isofemale lines from three populations.

D. aldrichi: *Experiment 3*

This experiment was done to repeat the earlier experiment for *D. aldrichi,* as testing subsequent to Experiment 2 had shown that optimum conditions for egg laying by *D. aldrichi* females were different from those for *D. buzzatii* (see Methods).

Analysis of variance of the number of eggs per disc gave similar results to those for *D. buzzatii* in Experiments 1 and 2, with significant effects for yeasts ($P < 0.001$), isofemale lines within populations ($P < 0.001$) and for yeasts × isofemale lines within populations ($P < 0.01$). Regression coefficients of the sensitivity index on total egg number for each replicate (Table 1) were not significant.

Analysis of the proportions of eggs on each yeast (Table 3, Fig. 8) gave significant effects for yeasts ($P < 0.001$) and for yeasts × isofemale lines within populations ($P < 0.05$).

As the twelve isofemale lines of *D. aldrichi* from Experiment 2 were included in Experiment 3, the results of the two experiments for these 12 lines could be compared. This comparison again addresses the ques-

TABLE 3. Analyses of variance of proportions of eggs per yeast (after Box-Cox-Bartlett transformation) for *D. aldrichi.*

Source of variation	Experiment 2				Experiment 3			
	df	Mean square	F†		*df*	Mean square	F†	
Population (P)	2	0.11489	0.657	(1)	2	0.00562	0.714	(1)
Isolines/P (I/P)	9	0.17486 (1)	0.392	(3)	18	0.00787 (1)	0.093	(3)
Yeast (Y)	3	8.69335	19.771***	(2)	3	2.45032	19.427***	(2)
Y × P	6	0.38026	0.865	(2)	6	0.17617	1.397	(2)
Y × I/P	27	0.43971 (2)	0.986	(3)	54	0.12613 (2)	1.490*	(3)
Residual	144	0.44608 (3)			252	0.08464 (3)		

† F-ratio with denominator mean square indicated in parentheses.
* $P < 0.05$; *** $P < 0.001$.

TABLE 4. Intraclass correlations (replicate repeatability) isofemale line heritabilities (H) and heritabilities of individual female preferences (h^2), estimated from analyses of multiple yeast preferences (proportions of eggs laid on each yeast).

Experiment	Intraclass correlation[1]	H	h^2
D. buzzatii			
1	0.158***	0.0093	0.0187
2	0.113***	0.0064	0.0128
3	0[2]		
4	0.203***	0.0126	0.0254
2 and 4	0.171***	0.0102	0.0205
3 and 4	0.243***	0.0158	0.0319
D. aldrichi			
3	0.044*	0.0023	0.0046

* $P < 0.05$, *** $P < 0.001$.
[1] Incorrect estimates of these intraclass correlations for experiments 1 and 2 were given in a preliminary report (Barker, 1990).
[2] V_B negative.

tion whether additional laboratory maintenance (9 generations versus 4) had resulted in any changes in yeast preferences of the lines. For the proportions of eggs on each yeast, the interaction experiment × yeast × isofemale lines within populations was not significant, so that there was no evidence for changes in oviposition preferences.

Heritability of Oviposition Site Preferences

For *D. buzzatii* in Experiments 1, 2, and 4 and for *D. aldrichi* in Experiment 3, the interaction for yeasts × isofemale lines within populations was significant (Tables 2 and 3), indicating differences among isofemale lines within populations in preferences for oviposition substrates. These differences must be predominantly genetic, as all lines in each experiment were grown and tested together under the same environmental conditions.

But how heritable are these preferences? Heritability of individual female preferences cannot be estimated directly from these data, but estimates can be derived from the repeatability of replicates. From the ANOVAS in Tables 2 and 3 and the combined analyses of Experiments 2 and 4, and 3 and 4 for *D. buzzatii,* the within line variance component for multiple yeast preferences (V_W) is the mean square for the interaction replicates × yeasts × isofemale lines within populations. The between lines variance component (V_B) is derived from this and the mean square for yeasts × isofemale lines within populations. The intraclass correlation (repeatability of replicates) is then:

$$V_B/(V_B + V_W).$$

In all these experiments, groups of 20 females were tested in each oviposition chamber, so that an estimate of isofemale line heritability (H) is given as (Hoffmann and Parsons, 1988):

$$V_B/(V_B + 20\ V_W).$$

The heritability of individual female preferences (h^2) is then estimated from the isofemale line heritabilty as follows (Hoffmann and Parsons, 1988):

$$h^2 = 1 \Big/ \left(\frac{1}{2H} - \frac{1}{4}\right).$$

TABLE 5. Intraclass correlations (t = replicate repeatability), isofemale line heritabilities (H) and heritabilites of individual female preferences (h^2), estimated separately for each yeast species.

Experiment	*C. sonorensis*			*P. cactophila*		
	t	H	h^2	t	H	h^2
D. buzzatii						
1	0.175	0.0105	0.0211	0.262**	0.0175	0.0353
2	0.043	0.0022	0.0044	0.109	0.0061	0.0122
3	0[1]	—	—	0.224	0.0142	0.0286
4	0.216*	0.0136	0.0274	0.135	0.0077	0.0155
2 and 4	0.044	0.0023	0.0046	0.221**	0.0140	0.0282
3 and 4	0[1]	—	—	0.482**	0.0444	0.0908
D. aldrichi						
3	0.056	0.0030	0.0060	0[1]	—	—

* $P < 0.05$, ** $P < 0.01$.
[1] V_B negative.

These estimates are given in Table 4, and while the intraclass correlations were generally highly significant, both isofemale line and individual female heritabilities were low.

However, it should be noted that these estimates relate to the multiple choices among five yeast species, and there could be genetic variation among isofemale lines for preferences for some species, but not for others. Therefore, the data were analyzed separately for each yeast species, i.e., for the proportions of eggs laid on each yeast, relative to the other four yeasts. In all analyses, data were transformed using the Box-Cox Bartlett transformation to ensure normality and homogeneity of variances for each isofemale line. ANOVA models and estimation methods for intra class correlations and heritabilities were as for the multiple yeast analyses, and results are shown in Table 5. For *D. aldrichi,* none of the intraclass correlations for individual yeast species were significant, and this one experiment using seven isofemale lines from each of three populations gives no evidence for heritable variation in preferences. On the other hand, *D. buzzatii* shows heritable variation for preferences for *P. cactophila, C. mucilagina,* and *Cr. cereanus* (with heritability of individual female preferences ranging up to 9%), but little or none for *C. sonorensis* and *Cl. opuntiae.*

Discussion

The most important result is the direct evidence for genetic variation for oviposition preferences on natural substrates, with significant variation among isofemale lines within populations in their patterns of oviposition on the five yeast substrates (Table 4).

For *D. aldrichi,* the patterns of yeast preferences were very similar in the two experiments (Fig. 4). This may not be surprising, as the isofemale lines derived from the same three populations for each experiment, and the four isofemale lines per population in Experiment 2 were included in the seven used in Experiment 3. On the other hand, *D. buzzatii* appeared to show differences in the patterns of yeast preferences in each experiment (Fig. 4). As the rank order preferences of the lines used in Experiments 2 and 4, and in Experiments 3 and 4 were not significantly different between experiments (Fig. 6), it must be concluded that the apparent differences among *experiments* for *D. buzzatii* result primarily from the differences in preference of the different isofemale lines included in each experiment.

However, there is a further factor relevant to the apparent differences among experiments and to the estimation of preferences within experiments. In each experiment, the variance of the proportions of eggs laid on each of the five yeasts was estimated as an index of sensitivity, where higher variance indicates increased expression of preferences. Regression coefficients of this variance on total egg number for each replicate, separately for each population and overall for each experiment (Table 1) were negative, with only two exceptions. That is, within populations or within experiments, there is a tendency for the expression of preferences

TABLE 5. Extended.

Cl. opuntiae			*C. mucilagina*			*Cr. cereanus*		
t	H	h^2	t	H	h^2	t	H	h^2
0[1]	—	—	0.143*	0.0082	0.0165	0[1]	—	—
0.054	0.0028	0.0056	0.217*	0.0137	0.0276	0.055	0.0029	0.0058
0[1]	—	—	0[1]	—	—	0[1]	—	—
0[1]	—	—	0.077	0.0041	0.0082	0[1]	—	—
0.000	0.0000	0.0000	0.298**	0.0208	0.0420	0.182*	0.0110	0.0221
0.214	0.0134	0.0270	0.425**	0.0357	0.0727	0.331*	0.0241	0.0488
0.105	0.0058	0.0116	0.046	0.0024	0.0048	0.156	0.0091	0.0183

to be reduced in the more fecund replicates or populations. Further, comparing experiments (for each species), the regression coefficients generally decreased as mean egg number per replicate increased, again indicating decreased sensitivity as average fecundity increased. These results suggest some degree of interference or required spacing between females at oviposition, so that when more females are ovipositing at the same time, some are forced to oviposit on yeasts that are intrinsically less preferred, i.e., density-dependent habitat selection (Rosenzweig, 1991).

Any such effect will reduce the precision of estimation of preferences. In addition, if females tend to oviposit in an aggregated manner, being attracted to other females or to sites where eggs are already laid, as has been found for *D. melanogaster* (Solar, 1968), then the proportions of eggs on each yeast will underestimate genetic preferences.

Both of these possible effects will cause the isofemale, and hence individual female, heritabilities to be underestimates. Nevertheless, significant genetic variation for oviposition preferences on naturally ocurring yeast species has been demonstrated.

For *D. buzzatii,* it is clear that this genetic variation for preferences relates to only three of the five yeast species. Although the heritabilities of individual female preferences for these three species were low (estimates of 0–9%, Table 5), there is potential for genotype specific habitat selection in natural populations.

However, is this oviposition preference a kind of habitat selection which maintains polymorphism? Resolution of this question will depend partly on who benefits—the female or her offspring. At the species level, *D. buzzatii* females differentiate among yeast species, but with similar rank orders for both oviposition and feeding (Vacek et al., 1985). But when these same females were identified to genotype (defined at seven polymorphic enzyme loci), significant differences among genotypes were detected for oviposition preferences, but not for feeding (Barker et al., 1986). That is, the feeding preferences observed at the species level were apparently similar for all genotypes, while the oviposition preferences were due, at least in part, to females of some genotypes preferring one yeast and other genotypes preferring other yeasts.

Oviposition site selection thus appears to be the primary mechanism, suggesting that the direct benefit is to the offspring. To substantiate this, it will be necessary to show that the offspring of a female preferring a particular yeast for oviposition have higher fitness on that yeast than on others. If this is true, there will also be an indirect benefit to the female through increased effective fecundity. Alternatively, the benefit to the offspring and to their mother may be simply density-dependent, as in Rausher's (1984) model of habitat preference.

There was little evidence for differentiation among populations from different localities. Only for *D. buzzatii* in Experiment 2 was the yeast × population interaction significant, due to different rank order preferences for the Injune Creek population, as compared with Borah and Westwood (Fig. 3). But when these same isofemale lines were retested in Experiment 4, this interaction was not significant. Jaenike (1989), studying oviposition preferences for mushrooms and tomatoes in *D. tripunctata*, obtained similar results, with significant variation among isofemale lines, but no population differentiation. He concluded that the species as a whole, rather than local populations, appeared to be the unit of evolution with respect to resource use. The lack of geographic differentiation may be due to similar selection regimes with respect to resource use across the species' range, or to extensive gene flow. For *D. tripunctata,* Jaenike (1989) favored gene flow as the more likely explanation. However, *D. buzzatii* is restricted to the cactus habitat, which occurs in discrete patches, and there is significant geographic differentiation for allozymes (Sokal et al., 1987). The five yeast species used here as resources are among the six most commonly isolated from cactus rots in Australia (Barker et al., 1984), and most likely occur throughout the cactus distribution. Thus similar selection is a more likely explanation of the geographic uniformity for oviposition site preferences.

Whatever the nature of this selection, genetic variation for oviposition preference is maintained within populations. The ques-

tion was raised previously if this oviposition preference is a kind of habitat selection which can maintain polymorphism. To this should be added—polymorphism for which loci? That is, will polymorphism be maintained only at loci determining the preferences or also at other loci? This question is fundamental in relation to attempts to explain the maintenance of genetic variation (Barker, 1990). A number of theoretical studies demonstrating that habitat selection can maintain polymorphisms are based on the assumption that individuals select a habitat in which to live, followed by differential viability selection in the different habitats (Taylor, 1976; Garcia-Dorado, 1986, 1987; Hedrick, 1990*a*). This is clearly not the same as habitat selection for oviposition, where selection by adults determines the habitat in which their progeny will develop. For this case, Rausher (1984) and Diehl and Bush (1989) have shown that polymorphism can be maintained at a locus determining the habitat preference, even with random mating and without the locus affecting fitness in different habitats. Further, Diehl and Bush (1989) found that significant disequilibrium can be maintained between unlinked loci affecting habitat selection and fitness in alternative habitats. Hedrick (1990*b*) also has studied a two locus model where the loci separately affect habitat selection and viability selection, and has considered the effect of varying selection intensity at the viability locus and varying recombination between the two loci. The probability of a stable polymorphism at the viability locus, particularly for low selection at this locus, was shown to be greatly increased only for tight linkage.

These models of Rausher (1984), Diehl and Bush (1989) and Hedrick (1990*b*) suggest that the genetic variation for oviposition preferences observed in the experiments reported here could be directly maintained by natural selection in natural populations. However, these preferences are likely to be polygenically based, as found by Jaenike (1987) for *D. tripunctata,* rather than due to a single locus. Clearly there is a need for modelling of polygenic habitat preferences, but assuming that results from the single locus habitat selection models hold in general for polygenic variation, polymorphism could be maintained at these loci affecting habitat selection. Linkage disequilibria between these habitat selection loci and other loci then could contribute to the maintenance of polymorphism at the latter. This expectation is consistent with the results of Barker et al. (1986) demonstrating habitat selection for oviposition sites by *D. buzzatii* females of different genotypes at seven electrophoretic loci.

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