Environmental effects and the genetics of oviposition site preference for natural yeast substrates in *Drosophila buzzatii*

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Habitat selection expressed as oviposition site preferences (OSP), is one component of the complex of behaviours of females seeking a place to oviposit. *Drosophila buzzatii* females lay their eggs in cactus necroses (rots), where the alternative oviposition sites are patches of adjacent or even partially mixed growing yeast species. The OSP exhibited by individual females is not absolute, but subject to environmental effects and the physiology of the fly, and may vary depending on the particular combination of yeast species present in a rot. Nevertheless, we have shown that OSP of *D. buzzatii* females is heritable, with evidence from variation among isofemale lines, direct estimation of heritability, generation means analysis and short term selection. Further, this genetic variation appears to be ubiquitous, polygenic and largely non-additive for all yeast species combinations. The consequences of such genotype-specific habitat selection for the maintenance of genetic variation are considered by an evaluation of our results in comparison with assumptions of models of habitat selection. As all assumed mechanisms of these models are apparently met, OSP for yeast species would seem to be a powerful force for the maintenance of genetic variation, and not only at loci affecting the choice of oviposition sites.

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Habitat selection by individuals within a species may influence many aspects of the evolutionary ecology of that species. For example, habitat selection may explain, or at least contribute to (i) the maintenance of genetic variation within populations (reviewed by HEDRICK 1986; JAENIKE and HOLT 1991), (ii) the evolution of host specialization (reviewed by JAENIKE 1990), (iii) the origin of species (RICE 1987; DIEHL and BUSH 1989) and (iv) determination of community structure (HOLT 1993). With regard to the first of these, VAN VALEN (1965) argued that heritable variation (single locus polymorphisms or polygenic variation) can be maintained in a population that inhabits a heterogeneous environment, if genetically different individuals vary in their ability to use different resources, and if they can select those which are most suitable. Subsequent theoretical analyses have shown that such genotype-specific habitat selection, with differential selection in the different habitats, can maintain stable polymorphisms (MAYNARD SMITH 1970; TAYLOR 1976; TEMPLETON and ROTHMAN 1981; GARCIA-DORADO 1986, 1987; HEDRICK 1990a,b). Under some conditions, genetic variation can be maintained at a locus affecting habitat choice, even when that locus does not affect fitness in the different habitats (RAUSHER 1984; DIEHL and BUSH 1989).

Several hierarchical stages may be important in habitat selection, from choice of a suitable broadly defined place in which to live (e.g., woodland vs. grassland), through choice of microhabitats within this, to choice of nutritional resources, and for oviparous species, choice of an oviposition site. For *Drosophila*, response to different oviposition sites may be most important in determining niche separation of species (CARSON 1971; SHORROCKS 1975), while within species, genotype-specific preferences for oviposition sites could be a major factor promoting habitat selection (BARKER 1992). Thus our focus here is on oviposition site preference (OSP), and on the consequences of such habitat selection for the maintenance of genetic variation.

Most *Drosophila* species are saprophagous, feeding on the decaying tissues of plants and fungi. Breeding sites are known for some species (BRNCIC 1983; JAENIKE and GRIMALDI 1983; LACHAISE and TSACAS 1983; HEED and MANGAN 1986; FOGLEMAN and HEED 1989; TAUBER and TAUBER 1989), but microhabitat variation within these sites generally is unknown. However, a major component of the diet of both larval and adult stages is the microbial community involved in the decay process. Thus habitat selection in drosophilids is at least partially tied to the cues, nutrition and deterrents produced by microorganisms during the necrotic process. Studies on

the microbial communities associated with the larval stages have shown that the pattern of similarity of the microbial communities of different hosts is congruent with the phylogeny of major groups of drosophilids (STARMER 1981). These observations support the view that divergence in habitat use is closely associated with the differences in the microorganism-larval affiliations. Although bacteria are likely to be the primary cause of decay (e.g., ALCORN et al. 1991), yeasts also are commonly found in the decaying tissues (see STARMER et al. 1991 for review of *Drosophila*–microorganism associations). Since the beginning of this century, yeasts have been recognized as important and essential components of drosophilid nutrition (DELCOURT and GUYENOT 1910; BAUMBERGER 1919). However, yeasts appear to be more important than just food. Recent research into the interactions of yeasts and drosophilids has revealed yeasts to be involved in:

(i) detoxification of harmful chemicals either present in the host tissues or produced during the decay process (STARMER 1982; STARMER et al. 1986),
(ii) producing chemical cues used by larvae or adults for finding food (FOGLEMAN 1982; FOGLEMAN and ABRIL 1990),
(iii) producing cues that signal suitable sites for oviposition (VACEK et al. 1985; BARKER et al. 1986),
(iv) altering pheromones produced by adults for the attraction of mates (BARTELT et al. 1989), and
(v) influencing the behaviour of adults during courtship and mating (COOK and COOK 1975; MCROBERT 1986; STARMER et al. 1988a).

The cactophilic *Drosophila*–yeast–cactus system provides an excellent opportunity for the study of habitat selection in terms of oviposition site preferences (BARKER 1990). Two cactophilic species, *D. aldrichi* and *D. buzzatii* (both in the *mulleri* subgroup of the *repleta* group), have colonized in Australia in association with species of *Opuntia* (prickly pear) cactus. Both species feed and breed in necrotic cactus cladodes and stems (cactus rots), and are specific to this niche. In Australia, cactus rots result primarily from the mining of cladodes by the phytophagous larvae of the moth, *Cactoblastis cactorum*, which was introduced as a biological control agent. The decaying cactus tissue is a nutrient-rich environment for the growth of microorganisms, which in turn supply essential nutrients for the *Drosophila*. The microorganisms rely on insects for dispersal, and the *Drosophila* serve this vectoring role (STARMER et al. 1988b). This feeding and breeding habitat of the *Drosophila* can be studied in terms of the potentially important microenvironmental resources of the rot microflora and factors such as rot chemistry (FOGLEMAN and ABRIL 1990), and it may be experimentally manipulated. In addition, both the *Drosophila* and the microorganisms can be grown and studied in the laboratory.

Previous studies of the cactophilic *Drosophila*–yeast–cactus system have considered the two related questions that often emerge in discussions of habitat selection (FUTUYMA and PETERSON 1985; VIA 1986; SINGER et al. 1988; THOMPSON 1988a)—(i) is there a genetic basis for habitat selection, and (ii) does genetically based behaviour correspond to some type of benefit to the organism? These previous studies have provided evidence for genotype-specific habitat selection in the *Drosophila buzzatii*–yeast–*Opuntia* cactus system (BARKER et al. 1986; BARKER 1990, 1992; BARKER et al. 1994). BARKER (1992) tested 60 isofemale lines from seven distinct populations for their oviposition responses when given a choice of five yeast species. Significant isofemale line heritability and heritability of individual female responses were found for three common cactus yeast species. A second set of oviposition preference tests on 90 isofemale lines obtained from nine different populations in eastern Australia also showed heritable responses for different yeasts as oviposition sites (BARKER et al. 1994). In a retest of 40 of these lines for oviposition responses to the yeasts *Pichia cactophila* (Pc) and *Cryptococcus cereanus*, the product-moment correlation coefficient between the mean (over replicates) proportion of eggs laid on *P. cactophila* in the original experiment and in the retest was 0.388 ($P < 0.05$), and the means for each line in the retest were substantially higher (average of all lines—0.874 vs. 0.640). However, a more concentrated cactus base was used for yeast growth in the retest, and a further experiment demonstrated that the proportion of eggs laid on Pc was significantly higher with this more concentrated cactus base (BARKER et al. 1994).

Four of the 90 lines, two with high and two with low oviposition responses for Pc, were selected for use in a field experiment, and were retested five generations after the first test. The results from this experiment were quantitatively different from the first test. Both high and low lines had decreased their response to Pc, but the relative order of the two types (high and low) remained the same and high versus low responses were significantly different. Again, however, there were differences in methods between the two experiments—in this case, the presence of a third yeast (*Clavispora opuntiae*) in the retest. Field tests of these four lines showed all to have higher responses to Pc under field conditions, yet the ranking of the high lines to the low lines was consistent

with the earlier laboratory results. Thus laboratory retests and field tests all show differences in the absolute magnitude of the oviposition response. These differences could have been due to genetic changes that occurred during maintenance in laboratory culture and/or changes in the test conditions (environment). The latter reason is most likely, indicating that oviposition responses are likely to show substantial environmental variation.

The aim of this paper is not to review habitat selection or the choice of hosts by ovipositing females, as a number of recent reviews are available (SINGER 1986; COURTNEY and KIBOTA 1990; JAENIKE and HOLT 1991; THOMPSON and PELLMYR 1991). Rather, we present a comprehensive experimental analysis of oviposition site preferences in one species of *Drosophila*. In the series of experiments reported here, we have tested the effects of various environmental variables on oviposition site preferences, evaluated effects of yeast combinations, carried out a detailed genetic analysis, compared larval and adult preferences, and compared female preference with progeny fitness.

MATERIALS AND METHODS

Fly stocks

(1) Westwood—derived from 51 females collected at Westwood, central Queensland (23°38′S, 150°08′E) in October 1987, and subsequently maintained as a mass population set up from their progeny (10 bottles per generation, 20 pairs of parents per bottle, with mixture of flies from different bottles in each generation).

(2) Dixalea—derived from 20 females collected in April 1991, at Dixalea, central Queensland (23°56′S, 150°17′E). After two generations as isofemale lines, two male and two female virgin progeny were collected from each line, and 10 bottles set up—each with four males and four females—two males of each of two lines, and two females of each of two other lines. Virgin progeny were collected from each bottle (approximately 50 pairs per bottle) and released into a large population cage. They were held for two days on a live *Saccharomyces cerevisiae* yeast paste in petri dishes, and 10 bottles were then inserted into the cage for 24 h. Subsequent generations were maintained as for the Westwood population.

(3) Isofemale lines—derived from single females collected in each of nine localities in October, 1989. These lines were used previously in the experiments of BARKER et al. (1994). Each line was set up in each generation in four vials (5 pairs of parents per vial), with parents transferred to a new vial after one day, and discarded one day later.

All stocks were maintained on an autoclaved sucrose–yeast–cactus medium (STARMER and BARKER 1986) seeded with live *S. cerevisiae*, and kept at 25°C, 65–70% relative humidity, and a 12:12 light cycle (light 0600–1800 h). All experimental flies (except experiments 2 and 9) were raised and stored under these same conditions. During laboratory maintenance, these stocks had not been exposed to cactophilic yeasts or bacteria.

Oviposition preference testing

All procedures for preparation of experimental flies, microorganism culture and setting up the oviposition test chambers were generally the same as those described in detail by BARKER (1992).

Each yeast species (together with a mixed bacteria community of six species to simulate the microorganism composition of rots in nature, BARKER 1992) was presented to the flies as 48 h cultures growing on the surface of a cactus homogenate medium. One homogenised batch of *O. stricta* was used to prepare this medium for any one experiment, and 30% *O. stricta* homogenate was used in all except experiment 7 (10%).

Oviposition chambers were 9 cm Petri dishes with a cotton-stoppered hole in the lid (for addition of flies) and a filter paper in the base moistened with 1 ml sterile water. Discs 1.6 cm in diameter were cut from the microbial culture slabs and placed around the periphery of each chamber. For pairwise choice of two yeast species, or for yeast monocultures vs. bicultures (experiment 5), two discs of each were equally spaced opposite each other, so that the yeasts alternated. Where three yeast species were tested, two discs of each were arranged similarly.

Experimental flies (except experiments 2 and 9) were stored from collection (with transfer to fresh vials every 2–3 days) to the day that they were set in the oviposition test chambers on the autoclaved cactus medium seeded with live *S. cerevisiae* (up to 10 pairs per 75 mm × 25 mm vial). During the morning of the day that oviposition preference tests were begun, the flies to be added to each chamber were taken at random from those stored, either using light CO_2 anaesthesia or without anaesthesia, and placed in vials containing 5 ml 1.5% agar. Between 1330 and 1500 h, with some variation between experiments, flies were added to the oviposition chambers. The chambers were then arranged in random order and placed in a dark incubator at 25°C, 65–70% relative humidity (except for experiment 1, where temperature was a treatment) by 1530 h at the latest. At the end of the oviposition test period, the chambers were put in a freezer at −15°C for about 20 min, and then transferred to a refrigerator (4°C) to prevent further

embryo development. The eggs on each disc were counted over following days.

Experiments 1–3 were designed to test effects of various environmental factors on oviposition preferences, where flies were given the choice between two yeast species—Pc and *Cryptococcus cereanus* (Crc).

Experiment 1—Effects of female density, presence or absence of males, oviposition periods and temperature

The Westwood mass population was used, and parents of experimental flies were at generation 76 of laboratory maintenance. Treatments were (i) density—1, 2, 4 or 8 females per chamber, (ii) males—present or absent, and equal to number of females when present, (iii) oviposition periods—5 or 29 h (as *D. buzzatii* females show a peak of oviposition in the late afternoon–early evening, these times allow for one or two peak oviposition periods), (iv) temperature—25°C or 28°C.

Parents of experimental flies were set up in 20 bottles, 20 pairs per bottle. Ten of these bottles were used to produce flies to be tested at 25°C, and 10 for 28°C. At peak emergence, progeny were collected separately from each bottle, and stored until used in the experiment, when they were nine days old. Twenty replicate chambers were set up for the one female/chamber treatments, and 10 replicates for the other densities. At each temperature, flies from one rearing bottle were used to set up one complete replicate (two replicates for one female/chamber). Thus any variation due to differences among rearing bottles is included in the replicate variance.

Experiment 2—Effects of female age and adult experience, and repeatability of preferences

The Dixalea mass population was used, and parents of experimental flies were at generation 27 of laboratory maintenance. The experiment was designed to determine effects of female age (3, 6 or 9 days), the repeatability of female preferences over four consecutive days, and to retest effects of adult experience of yeast species on oviposition preferences (HEDRICK et al. 1990).

Parents of the experimental flies were taken from the stock population, and set up in 120 vials at 3 pairs/vial. These parents were discarded one day later, and on day 7, plastic sleeves (with outside diameter equal to the inside diameter of vials) inserted into the vials as pupation sites. The sleeves were removed on day 11, and attached pupae were sterilized by immersion in Chlorox bleach solution (4%) for 10 min, then transferred twice through 0.7% NaCl solution (5 min each), and rinsed in sterile water. Each sleeve was then inserted into a cactus medium vial that had been seeded two days previously with one of the experience treatments, viz. (i) *S. cerevisiae* as control, (ii) *P. cactophila*, (iii) *Cr. cereanus*, or (iv) *P. cactophila* and *Cr. cereanus* (each seeded onto one side of a microscope slide inserted into the medium), and kept at 25°C (30 vials/treatment). Eclosing adults thus were exposed immediately to an experience treatment. Two days after peak emergence, all flies required for the experiment were collected, and stored (6 pairs from each treatment vial) in medium vials seeded with the same yeast(s) as that onto which they had eclosed. Until used in the experiment, these flies were transferred to fresh medium vials with the same yeast(s) at least every two days.

When the experimental flies were 3 days old, one pair was aspirated from each storage vial (i.e. 30 replicates) to an agar vial, and 4–5 h later transferred to an oviposition chamber. The next morning (0900–1000 h), flies were collected from the oviposition chambers under very light CO_2 anaesthesia, and stored in 1.5% agar vials. A further set of 120 oviposition chambers were prepared during this time, and the flies were transferred to these, and returned to the incubator by 1030 h. This procedure was repeated twice more, so that each female was tested for oviposition preference over four consecutive days. The same procedure was followed for flies 6 days old at the start of testing, and also 9 days old. Thus for each experience treatment, the 30 replicate pairs of flies at each initial age had been reared and stored in different vials, but each parental vial provided one pair of flies for each of the three initial ages.

Experiment 3—Effects of yeast growth conditions

Both the Westwood and Dixalea mass populations were used, and parents of experimental flies were at generations 96 and 37, respectively of laboratory maintenance.

Although all procedures for yeast growth and preparation of the oviposition choice disks were standardised, the proportion of eggs laid on *P. cactophila* varied between comparable experiments (both those reported here and in BARKER et al. 1994). It was thought that this variation may have been due to poorer growth of *Cr. cereanus* on the cactus homogenate in some experiments. Thus two *Cr. cereanus* growth conditions were used as treatments, (i) standard (as in previous experiments)—sample taken from the stock culture, grown for 2 days at 25°C on yeast–maltose–agar (YMA) plates, and then transferred to 30% cactus homogenate for two days growth at 25°C, and (ii) sample taken from stock culture, and put through three transfers at 2 day intervals onto YMA plates at 28°C, and then transferred to the cactus homogenate as for treatment (i).

Again females were tested over three consecutive days, using the same procedures as in experiment 2.

Parents of experimental flies were taken from the stock populations, and 40 vials set up for each population (3 pairs/vial). Progeny from each vial were collected on the day of peak eclosion, and stored separately (5 pairs/vial). Forty replicate oviposition chambers (1 pair of flies/chamber) were set up for each of the four treatments (2 populations × 2 Crc growth conditions). Again the flies for each replicate had been raised and stored in different vials, and therefore were not subjected to common environmental effects. All flies were 9 days old when first put in the oviposition chambers.

Experiments 4 and 5 examined oviposition preferences for five yeast species—in two-way and three-way choice combinations, and in monocultures versus bicultures, respectively. The yeast species (*Candida sonorensis* (Cs), *Pichia cactophila*, *Clavispora opuntiae* (Clo), *Pichia barkeri* (Pb), *Cryptococcus cereanus*), are the five most abundant in cactus rots in Australia (BARKER et al. 1984), and *D. buzzatii* females show a wide range of oviposition preferences for them (VACEK et al. 1985). In both experiments, the flies were left in the chambers for one oviposition period.

Experiment 4—Two-way and three-way choice for five yeast species

The flies used comprised four isofemale lines from each of nine populations; these lines being a subset of the 10 lines from each population used by BARKER et al. (1994). These lines were at generation 35 prior to initiation of the experiment. The five yeast species were used in all 10 two-way choice combinations, and all 10 three-way choice combinations. With nine populations, four isofemale lines per population and 20 yeast species combinations, a total of 720 oviposition choice chambers were set up at any one time. This was repeated to give four blocks in time: with blocks 1 and 2, and 3 and 4 each set up seven days apart, and with 21 days between blocks 2 and 3. Procedures were identical for each block.

For each line in each block, adults from stock cultures were stored at 10 pairs/vial for 24 h, then set up in two vials, 3 pairs/vial for 24 h. Progeny emerging from these vials were collected (30 pairs/line), and stored at 10 pairs/vial. In each block, when these stored flies were 8–9 days old, one male and one female was taken at random to set up each of the 20 yeast combination oviposition chambers for each line.

Experiment 5—Effects of mono- versus bicultures of yeast species

The experiment was set up in two blocks four weeks apart, using the Dixalea mass population at generations 40 and 41 of laboratory maintenance. The five yeast species were used in all 10 two-way choice combinations, and all 20 combinations of mono- versus bicultures (e.g., each of Cs and Pc in monoculture set up in oviposition chambers with Cs + Pc growing together on the same discs). In each block, 25 replicate chambers were set up for each yeast combination, i.e. 750 chambers/block.

Flies collected from the stock population were stored for 24 h—60 vials, 6 pairs/vial, then set up in 25 bottles, 10 pairs/bottle for 24 h. Progeny from each bottle were collected separately, stored 6 pairs/vial in six vials until used in the experiment, when they were 8–9 days old. On the morning that the oviposition chambers were set up, flies collected from each of the 25 bottles were set up as 30 single pairs in agar vials, and later used in one set of the 30 treatments. Thus all flies for any one replicate came from the same rearing bottle.

Experiment 6—Heritability of oviposition preferences

A full- and half-sib design was set up to estimate the heritability of oviposition preference, using the Dixalea mass population. The experiment was done in five blocks, with each block set up to have 15 sires, three dams/sire, and five female progeny/dam tested for oviposition preferences. For these matings, 20 sires were initially set up, each with six dams. After three days, the sires were discarded, and dams placed individually in vials. Eight virgin progeny were collected from each dam, and at this time, numbers were reduced to the 15 sires, three dams/sire. At the same time that dams were transferred to individual vials, six bottle matings (10 pairs/bottle) were set up from the random mated stock population. Male progeny were collected from these bottles. Virgin female progeny from each dam were stored with these random males (4 vials—each with 2 females and 4 males) for six days, with transfer to fresh vials every two days. Each female was then placed in an individual vial with two random males, and transferred to fresh vials two days later. Two days later (when the test females were 10 days old) the oviposition test chambers were set up. In block 1, a single test female was added to each chamber, while in blocks 2–5, one male was put with each female. The sires and dams used in block 1 were generation 3 of laboratory maintenance, while those in blocks 2–5 were generations 6, 8, 10 and 21, respectively.

Test females were given the choice of two yeast species—*Pichia cactophila* and *Cryptococcus cereanus* as oviposition substrates, on each of three consecutive days.

On the first day of testing, each female (plus one male in blocks 2–5) was transferred without anaesthesia to a vial containing 5 ml 1.5% agar, and 4–5 h later transferred to an oviposition chamber. On the morning of the following day, flies were removed from the oviposition chambers under light CO_2 anaesthesia to 1.5% agar vials. Flies were transferred to new oviposition chambers (prepared that morning) 4–5 h later, and these procedures repeated the next day. The following morning, flies were removed from the chambers and discarded. Thus the record for each female was the numbers of eggs laid and the proportion of these that were laid on Pc, for each of three consecutive days.

Because both egg number and proportion on Pc might be expected to vary from day to day, analyses were done on the combined data for days 1 and 2, for days 2 and 3, and for days 1, 2 and 3, as well as for each of the three days. For the combined data, egg number was the sum over the two (or three) days, and the proportion of eggs laid on Pc was the summed number of eggs on Pc as a fraction of total eggs.

Experiment 7—Generation means analysis

Four isofemale lines were used, chosen on the basis of previous results for oviposition choice of the yeast species *P. cactophila* and *Cr. cereanus*, as two that showed high preference for Pc (T9 and IG2), and two that showed low preference for Pc (T3 and MB6). These lines originated from three different natural populations—Theodore (T), Mulambin Beach (MB) and Isla Gorge (IG), and had been maintained in the laboratory for 46–48 generations before use in this experiment. All four combinations of high and low Pc preference lines were studied, with appropriate matings done separately for each pair of lines to produce 14 different genotypes of females (Table 1) for simultaneous testing of oviposition preferences. Individual females were tested, and in order to obtain large numbers of replicates, the mating and testing procedure was repeated seven times (blocks). At least two of the line combinations were included in each block, and each line combination was tested in a total of four or five of the blocks. For each line combination in each block, initial matings were set up in six vials, 3 pairs/vial for each line. Virgin progeny from these matings were used to produce F_1 (six vials, 3 pairs/vial for each of the reciprocal crosses) and pure line progeny (eight vials, 3 pairs/vial for each line). Virgin progeny again were collected and used to set up matings to produce all 14 genotypes.

The two yeast species used (*P. cactophila* and *Cr. cereanus*) were chosen on the basis of previous experiments (BARKER 1992; BARKER et al. 1994) as ones for which *D. buzzatii* showed heritable variation for oviposition preferences. Each yeast species was presented to the flies as 48 h cultures growing on the surface of a 10% cactus homogenate medium.

In blocks 1–3, 10 replicate oviposition chambers were set up for each genotype, and 18 replicates in blocks 4–7. Within each block, the matings to produce the experimental flies of each genotype were set up in 10 or 18 replicate vials (2 pairs of parents/repli-

Table 1. *The genotypes produced in crosses between lines showing high (H) or low (L) preference for oviposition on P. cactophila, and the expected percentage of genes from the H line in females of each genotype*

Category	Parents	Offspring	Offspring genotype code	% genes from H line in female offspring	
				X chromosomes	Autosomes
Parents	H	H	1	100	100
	L	L	2	0	0
F_1	H♂ × L♀	HL	3	50	50
	L♂ × H♀	LH	4	50	50
F_2	HL♂ × HL♀	HLHL	5	25	50
	LH♂ × LH♀	LHLH	6	75	50
Backcrosses to H♀	HL♂ × H♀	HLHH	7	50	75
	LH♂ × H♀	LHHH	8	100	75
Backcrosses to L♀	HL♂ × L♀	HLLL	9	0	25
	LH♂ × L♀	LHLL	10	50	25
Backcrosses to H♂	H♂ × HL♀	HHHL	11	75	75
	H♂ × LH♀	HHLH	12	75	75
Backcrosses to L♂	L♂ × HL♀	LLHL	13	25	25
	L♂ × LH♀	LLLH	14	25	25

Table 2. *Numbers of replicates of each genotype for each of the four line combinations*

Genotype code[a]	Line combination			
	T9/T3	T9/MB6	IG2/T3	IG2/MB6
1	73	88	102	96
2	94	97	113	98
3	52	43	64	60
4	53	52	63	60
5	47	42	64	60
6	43	38	60	58
7	41	46	62	57
8	40	39	61	50
9	51	49	66	62
10	52	44	64	61
11	43	43	64	62
12	39	46	61	63
13	52	53	64	55
14	52	49	60	56

[a] See Table 1

cate) on autoclaved cactus medium seeded with live *S. cerevisiae*. Three pairs of progeny from each vial were collected from the two days of peak emergence and stored in fresh vials of this same medium for two days. On the day before preference tests were set up, one pair (one male, one female as the experimental unit for oviposition preference testing) was taken at random from each vial under light CO_2 anaesthesia and stored on fresh cactus medium. Thus the replicate pairs tested for each genotype were reared in separate vials, and did not share a common environment, either as larvae or as adults. The following morning, the oviposition chambers were prepared, and the single pairs of flies to be added to each chamber were transferred without anaesthetization to vials containing 5 ml of 1.5% agar. Between 1330 and 1430 h, these flies (then 3–5 days old) were added to the oviposition preference chambers, and left for two oviposition periods.

Data from each of the four isofemale line combinations were analyzed separately, but all replicates for each parental line within each block were included in each line combination. For example, if the combinations T9/T3 and T9/MB6 were both run in a given block, then all replicates of T9 were included in both analyses. In all analyses, replicates with five or less eggs laid were deleted, and final numbers of replicates are given in Table 2. Results for each replicate were expressed as the proportion of eggs laid on *P. cactophila*, but because of bimodality in the data, with a number of replicates showing 0 or 100 % of eggs on *P. cactophila*, residuals deviated significantly from normality for all four line combinations. Apart from the excess of observations at the extremes of 0 and 100 %, the distributions were approximately normally distributed. The angular transform of JOHNSON and KOTZ (1969) quoted by SOKAL and ROHLF (1981, p.428) improved the fit to normality, and was used for all analyses.

Initial analyses were done using ANOVA (Proc GLM, SAS 1989), with blocks and genotypes as fixed effects. Although the genotypes effect was significant for all four line combinations, the specific objective was to make pre-planned comparisons testing for maternal effects, X chromosome or autosomal effects on oviposition preferences. These comparisons are as follows, where the genotype with expected higher preference is written first (see Table 1):

Maternal effects	−4 vs. 3,
X chromosome effects	−6 vs. 5, 8 vs. 7, 10 vs. 9,
Autosomal effects	−1 vs. 8, 9 vs. 2, 7 vs. 3, 7 vs. 4, 3 vs. 10, 4 vs. 90, 5 vs. 13, 5 vs. 14, 11 vs. 6, 12 vs. 6, 7 vs. 10.

Because the block × genotype interaction was significant in the ANOVA of each line combination and the number of replicates per genotype varied substantially (Table 2), each comparison was tested using the least squares means and their standard errors computed by the SAS GLM procedure. Although this ANOVA approach is robust to non-normality, each comparison was also analyzed by fitting log linear models (program BMDP 4F—DIXON 1992) to the proportion of eggs laid on *P. cactophila* in each replicate.

Given significant differences between genotypes in these comparisons, joint scaling tests (MATHER and JINKS 1982) were used to derive estimates of additive and dominance effects (sex-linked and autosomal), maternal effects and digenic epistatic effects, by sequentially fitting a number of models. This was done separately for each of the four line combinations, using the least squares means and their standard errors for appropriate genotypes, as follows:

(i) Model with autosomal and sex-linked additive and dominance effects, using 11 genotype means (MATHER and JINKS 1982, p. 295),
(ii) Model with autosomal and maternal additive and dominance effects, using nine genotype means (MATHER and JINKS 1982, p. 302),
(iii) Model with autosomal additive, dominance and digenic epistatic effects, using six genotype means (MATHER and JINKS 1982, p. 94),
(iv) Fitting the best model, including only significant effects from the above three models, using six genotype means.

In all cases, assuming normality, goodness-of-fit of the arcsine transformed genotype means to the model was tested with the chi-square statistic derived by HAYMAN (1958), using $P < 0.05$ as the level of significance.

A joint scaling test was used also to analyze the variances of the six genotypes (both parents, F_1, F_2 and back-crosses) under an additive model (HAYMAN 1960; LANDE 1981), and to estimate the minimum number of effective factors contributing to the difference between the parental isofemale lines (LANDE 1981).

Experiment 8—Selection for oviposition preferences

The genetic basis of oviposition preferences was tested further by determining response to selection. Using the yeast species Pc and Crc, selection was done for increased and decreased Pc preference. The base population derived from 27 females collected at Isla Gorge (25°14.84′S, 149°56.46′E), with their progeny mixed over two generations, and then mixed and set in eight bottles, 20 pairs/bottle to form a mass population which was maintained at this size in subsequent generations.

The isofemale lines also were maintained separately, and a preliminary experiment done to determine genetic variation among these lines. At generation 7 of laboratory maintenance, 23 vials were set up for each line, 3 pairs/vial. At peak emergence, progeny from each vial were collected separately and stored 3 pairs/vial. When these flies were five days old, one pair was taken from each of 22 vials per line, and oviposition chambers set up for each pair (two discs each of Pc and Crc, alternating). Flies were removed the next morning.

After 15 generations of laboratory maintenance of the base population, 15 pairs of virgin progeny were collected from each bottle, mixed and stored (sexes separate, 10/vial) in vials inoculated 48 h previously with a mixed suspension of Pc and Crc. These flies were used to initiate three replicate selection lines. When 4–5 day old, they were set in six vials, five pairs/vial (on Pc/Crc mixed). Four days later, 75 single pairs were put in oviposition preference chambers (two discs each of Pc and Crc, alternating). The following morning, flies were removed under light CO_2 anaesthesia, stored in agar vials for about 5 h, and then set in fresh oviposition plates. Again the next morning, flies were removed and stored in agar vials. Eggs on all discs were counted, and selection performed. In generation 0, the 75 pairs were split at random into three sets of 25 to initiate the three replicate lines. In each set, pairs were selected on the proportion of eggs laid on Pc, the five highest to initiate selection for increased Pc preference (Pc^+), the five lowest to initiate selection for decreased Pc preference (increased Crc preference—Crc^+). Selected pairs were put individually in vials inoculated with both Pc and Crc to reproduce.

In following generations, eight pairs of virgin progeny were collected from each parental pair. At five days old, all males were mixed, and from each parent pair, five females were set with five random males in one vial, and two females with two random males in a second vial (these latter as spares). At nine days old, five sets (each from different parental pairs) of five pairs were set as single pairs in oviposition preference chambers and tested over two days as in generation 0. From G. 1, selection was based on an index of individual and family mean preference. The index was derived as follows (LUSH 1947):

$$\text{Index (I)} = P + [(r - t)/(1 - r) \cdot (n)/(1 + (n - 1)t]P_f$$

where

P = individual preference
P_f = family mean preference
$r = 0.5$ (genetic relationship of full-sibs)
n = family size
t = intra-class correlation between family members $= 0.5\ h^2$
h^2 = heritability

For $h^2 = 0.05$, $I = P + 4.318\ P_f$

$h^2 = 0.10$, $I = P + 3.75\ P_f$

Thus the index used was $P + 4P_f$.

In each Pc^+ line, the five pairs with highest index were selected, while in each Crc^+ line, the five with lowest index were selected. Selection was continued for five generations. Throughout, flies were stored, and progeny from selected pairs raised in vials inoculated with both Pc and Crc.

Experiment 9—Adult performance on different yeasts, and comparison of larval and adult preferences

This experiment compared the performance on *P. cactophila* and *Cr. cereanus* of isofemale lines of known preferences for these yeast species, and compared larval and adult preferences. The lines used were the same four as in Experiment 7, i.e. two with high preference for Pc (T9 and IG2), and two with low preference for Pc (T3 and MB6). These lines were at generation 63 of laboratory maintenance before being expanded over two generations to produce large numbers of flies for egg collection. Eggs were collected between 1500 and 2000 h on three occasions (three and four days apart), sterilized the next day (STARMER and GILBERT 1982), and 0–2 h old larvae

collected from 0800 h the following morning. Larvae from the first two collections were put at 40/vial into vials containing 8 g autoclaved, homogenized *O. stricta* that had been inoculated two days previously with 100 µl of an opaque, milky suspension of either Pc or Crc and then kept at 25°C. For collection 1, 20 vials were set up per yeast per line (total of 160 vials), while for collection 2, numbers of vials/yeast/line varied from 26 to 33 (with more set on Crc—total of 228 vials).

Emerging adults were collected daily, sexed and counted. Those from collection 1 were accumulated in empty vials until emergence ceased, dried at 60°C for at least 5 days and then weighed. Data available from each vial were thus average developmental times for males, females and overall, larvae-to-adult viability, sex-ratio (proportion of males) and average weights for males, females and overall (mean of male and female average weights). Adults emerging from collection 2 vials were scored in the same way, but were not dried. Instead, flies emerging on the two days of peak emergence were stored in vials yeasted with either Pc or Crc (the same as that on which they had developed) for two days, and then used to measure daily fecundity to 15 days old. Single pairs were placed in bottles with a plastic spoon containing cactus agar (10% *O. stricta* homogenate, 1.5% agar) smeared with a drop of Pc or Crc suspension, and transferred daily to fresh spoons. Thirty pairs were set up for each of the eight line-yeast combinations, except for IG2 on Crc with 21. Two measures of fecundity were calculated—(i) average number of eggs/day of female life, and (ii) average number of eggs/test day (i.e. including survival as well as fecundity).

Larvae from collection 3 (0–2 h old) were used to test their attraction to the two yeasts Pc and Crc. For each line, 10 replicates were set up with 20 larvae placed into the centre of a 9 cm petri dish containing cactus agar (15% *O. stricta* homogenate, 1% agar). Around the periphery of each dish, four 1 cm diameter patches of yeast (two Pc, two Crc alternating) had been inoculated 24 h previously and the dishes then kept at 25°C. The numbers of larvae in, or in contact with, each yeast patch were counted 5, 15, 30, 45 and 60 min after larvae were placed in the dish. All replicates were run on one morning, with the four lines for each replicate set up at 20 s intervals, and replicates at 20 min intervals.

In a separate experiment, third instar larvae were tested. Larvae were collected, washed twice in 0.7% saline, and tranferred to 1.5% agar plates before transfer to the test dishes. For each strain, six replicates were set up at each of two times (one week apart), and the numbers of larvae in, or in contact with, each yeast patch were counted at 15 and 30 min after larvae were placed in the dish.

Statistical analyses

Except for experiments 6 and 7 (see above), analysis of variance (SAS GLM procedure, SAS 1989) was used to analyze results for the number of eggs laid in each oviposition chamber, and for the proportion of eggs laid on a particular yeast species. In all experiments, no eggs were laid in some of the oviposition chambers. In analyses of egg number, these were treated as zero data points and analyses were fully orthogonal. However, they lead to missing observations in analyses of proportions of eggs laid on a particular yeast, so that a Satterthwaite approximation was used to construct some of the F ratio tests. Type III sums of squares were used for all F-tests, and appropriate ratios of mean squares for F-tests involving random effects were obtained using the TEST option of the SAS GLM procedure. Normality of residuals in all ANOVA was tested using the Wilk-Shapiro W statistic (SAS UNIVARIATE procedure, SAS 1985), and homogeneity of variances was evaluated by Bartlett's test (SOKAL and ROHLF 1981). Unless otherwise indicated, the distributions of residuals were normal ($P_W > 0.05$) and variances were homogeneous ($P_{\chi^2} > 0.05$).

RESULTS

Experiment 1—Effects of female density, presence or absence of males, oviposition periods and temperature

Total number of eggs counted in the 400 chambers was 65,891 (mean eggs/chamber = 165, with standard deviation = 142.4). The mean proportion of eggs laid on Pc (PropPc) was 0.8938, with many values of 1.0 (particularly for density = 1), and with variances tending to increase with means. No eggs were laid in seven replicates of the density = 1 treatment, and for analysis of PropPc these missing observations were replaced by the mean of the other 19 replicates. PropPc was arcsine transformed for analysis, but residuals still were not normally distributed ($W = 0.870$, $P < 0.001$). For the average number of eggs per female in each oviposition chamber, there was a strong dependence of variance on mean, which was eliminated by a square root transformation, but residuals were not normally distributed ($W = 0.825$, $P < 0.001$).

Analyses of variance (Table 3) showed all main effects were significant for both PropPc and average eggs/female, and the treatment means (Table 4) show that for density, period and temperature effects, PropPc decreased as total eggs increased (Note for density, values in Table 4 are average eggs/female). For the male effect, however, both PropPc and eggs were higher when males were not present.

The main effect of density suggests interference among females at the oviposition site. That is, as density increases, some females do not gain access to the preferred Pc sites (at least some of the time), and consequently oviposit on the less preferred Crc. Such female–female interference is indicated also by the reduced average eggs/female at densities 4 and 8.

The male effect, however, with significantly more eggs/female and higher PropPc when males were absent, indicates male–female interference. The nature of such interference is shown by the significant density × males interaction for PropPc (Fig. 1), where with males present, there was a greater decrease in PropPc as density increased than where males were absent. However, the density × males interaction was not significant for eggs/female. That is, at higher densities, with more females likely to be on the preferred yeast at any time, male activity disturbs these females, which then lay on the less preferred yeast.

Period was significant for both PropPc and eggs. PropPc was less for two periods than for one, while average eggs/female for two periods were more than twice that for one period. Again, the significant density × period interaction for both PropPc and eggs points to the nature of the effects on oviposition and female preference. For one period, there were no significant differences among average eggs/female at the four densities, although PropPc decreased as density increased, i.e. female–female interference at the preferred oviposition site. However, when females were allowed two oviposition periods, there was a greater decrease in PropPc as density increased than for one period, and average eggs/female significantly decreased from densities 1 and 2, to 4, and to 8. The proportionate increase in average eggs/female from one period to two periods decreased as density increased, but for total eggs laid, numbers increased as density increased (average total eggs/chamber at densities 1, 2, 4, 8 for two periods were 102, 192, 296 and 463). That is, the greater decreases in PropPc and in average eggs/female as density increased for two periods were likely due to an effect of egg density on the oviposition surface.

The significantly lower PropPc at 28°C as compared with 25°C may be partly an egg density effect (eggs/female significantly higher at 28°C than at 25°C) or due to increased female–female interference on the preferred yeast at 28°C. However, significant interactions involving temperature for PropPc were only for period × temperature and for eggs only for density × temperature, suggesting possible direct effects of temperature on female oviposition preferences.

Experiment 2—Effects of female age and adult experience, and repeatability of preferences

With 4 conditioning treatments × 3 initial female ages × 4 consecutive test days, and 30 replicates, experiment 2 used 1440 oviposition chambers. For 88 of these, no eggs were laid, thus there were 1352 observations for the proportion of eggs laid on Pc. As in

Table 3. *Analyses of variance of arcsine transformed proportion of eggs laid on P. cactophila, and square root transformed average eggs/female in Experiment 1*

Source	df	Arcsine (PropPc)		SqRt (Eggs/female)	
		Mean squares	F	Mean squares	F
Density (D)	3	10102.33	69.86***	38.678	14.22***
Males (M)	1	2614.18	18.08***	15.516	5.71*
Period (P)	1	10535.57	72.85***	1005.956	369.96***
Temp (T)	1	714.54	4.94*	34.837	12.81***
D × M	3	425.41	2.94*	2.535	0.93
D × P	3	646.91	4.47**	19.183	7.05***
D × T	3	350.08	2.42	7.199	2.65*
M × P	1	311.88	2.16	1.200	0.44
M × T	1	27.95	0.19	0.922	0.34
P × T	1	1891.14	13.08***	2.388	0.88
D × M × P	3	639.63	4.42**	1.469	0.54
D × M × T	3	102.38	0.71	6.041	2.22
D × P × T	3	237.60	1.64	4.221	1.55
M × P × T	1	36.01	0.25	1.031	0.38
D × M × P × T	3	119.63	0.83	3.201	1.18
Error	368	144.61		2.719	

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 4. *Treatment means for PropPc and average eggs/female (back-transformed) in Experiment 1*

Density		1	2	4	8
	PropPc	0.994	0.977	0.904	0.800
	Eggs	58.9[a]	60.5[a]	49.7	40.5
Males		Present	Absent		
	PropPc	0.938	0.969		
	Eggs	50.2	56.6		
Period		1	2		
	PropPc	0.982	0.914		
	Eggs	30.9	82.0		
Temperature		25	28		
	PropPc	0.965	0.942		
	Eggs	49.3	57.6		

[a] Not significantly different

experiment 1, PropPc had many values of 1.0, and was arcsine transformed, but arcsine (PropPc) still showed significant deviation from normality. The fit to normality was improved (but still significant deviation—$W = 0.693$, $P < 0.001$) by deleting replicates where ≤ 5 eggs were laid (17 replicates). This reduced data set was used in all analyses of PropPc. The distribution of residuals for the number of eggs laid in each oviposition chamber did not deviate from normality, and all 1440 observations (including zero eggs) were included in the analyses.

Analyses of variance, with treatment, age and day as fixed effects (Table 5), showed for the proportion of eggs laid on Pc significant effects of day ($P < 0.05$) and for the second order interaction ($P < 0.01$). The proportion of eggs laid on Pc was significantly higher on day 1 (0.83) than on the three later days (0.79). For the number of eggs laid in each oviposition chamber, all main effects and first order interactions were significant. Egg number was highest for the Crc and Pc + Crc treatments (58.5 and 59.5, respectively), intermediate for *S. cerevisiae* (55.1) and lowest for Pc (51.9); it decreased from initial age 3 to 6 to 9 (61.5, 55.3 and 51.9, respectively), and was lowest on day 2 (45.4 as compared with 62.4, 58.3 and 59.0 on days 1, 3 and 4). Egg number decreased with increasing initial age of females from 3 to 6 to 9 days for all except the Pc + Crc treatment (significant treatment × age interaction). For females initially 3 days old, egg number tended to increase over the four test days, while for 6 and 9 day old females, egg number decreased markedly on day 2, and increased to somewhat less than day 1 numbers on days 3 and 4.

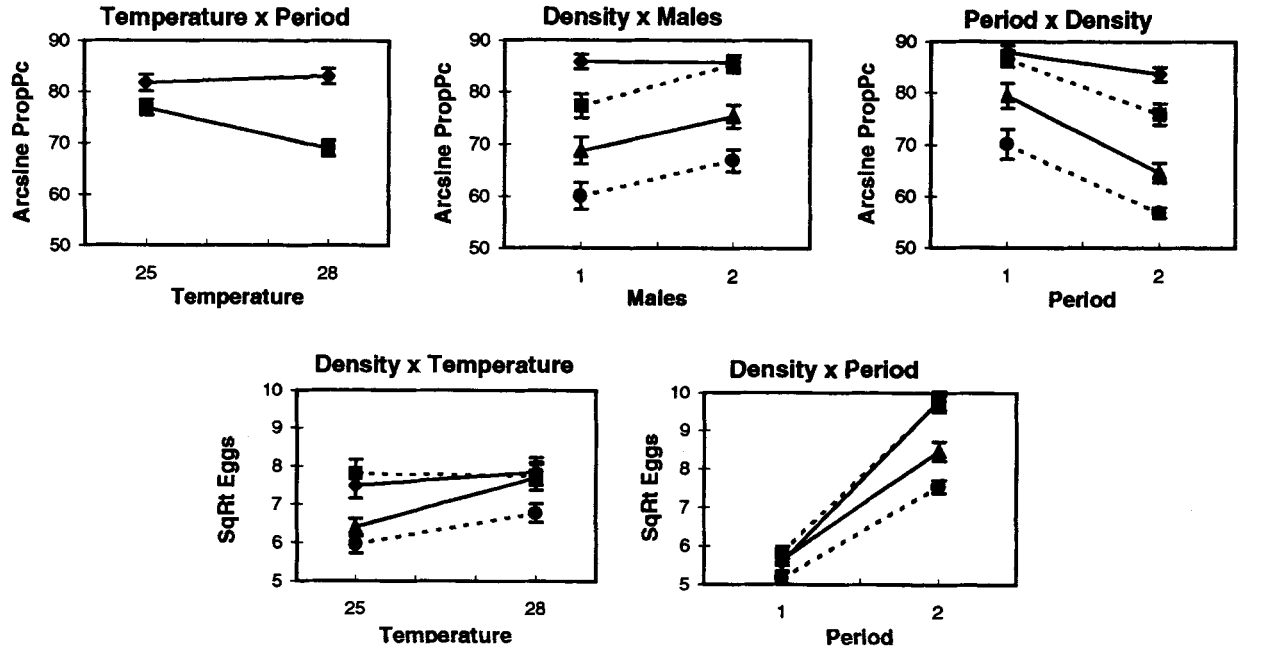

Fig. 1. Significant interaction effects in Experiment 1. For density, ◆ = 1, ■ = 2, ▲ = 4, ● = 8, and for males, 1 = males present, 2 = males absent.

Correlation coefficients among days (as a measure of day-to-day repeatability) for arcsine (PropPc) and egg number (Table 6) show some variability with initial female age (particularly for arcsine (PropPc)), but overall estimates for arcsine (PropPc) are significant for days 2, 3 and 4. For egg number, overall correlations are significant for all except days 3 and 4.

Repeatability over days also was estimated from the variance components (calculated using the VARCOMP procedure of SAS) for between individuals within treatment × age, and for between days within individuals (BECKER 1984), as 0.138 ± 0.024 for arcsine (PropPc) and 0.128 ± 0.026 for egg number.

Experiment 3—Effects of yeast growth conditions

Of the 480 oviposition chambers (2 populations × 2 Crc treatments × 3 days, and 40 replicates), no eggs were laid in 59 and ≤ 5 eggs in a further nine. Again PropPc had many values of 1.0, and arcsine transformed PropPc showed a closer fit to normality after deleting values based on ≤ 5 eggs, but as in experiment 2, the deviation from normality was still significant ($W = 0.655$, $P < 0.001$).

Analyses of variance showed no significant effects for arcsine (PropPc), while for egg number, there were significant effects for population ($P < 0.001$), Crc treatment ($P < 0.01$) and population × day ($P < 0.01$). Egg numbers were higher for Westwood than for Dixalea (55.7 vs. 44.8), and for Crc growth at 25°C than at 28°C (53.8 vs. 46.6). For Dixalea, egg numbers increased from day 1 to day 2 and then decreased to day 3, while for Westwood, egg numbers were highest on day 1 and then similar on days 2 and 3. Repeatability over days (estimated as for Experiment 2) was 0.123 ± 0.057 for arcsine (PropPc) and 0.423 ± 0.049 for egg number.

Table 5. *Analyses of variance of arcsine transformed proportion of eggs laid on P. cactophila, and number of eggs laid/chamber in Experiment 2*

Source	df	Mean squares	
		Arcsine (PropPc)	Eggs/chamber
Treatment (T)	3	59.745	4342.86***
Age (A)	2	860.260	11370.34***
Day (D)	3	1253.922*	20221.35***
T × A	6	647.316	2285.01**
T × D	9	227.483	1646.93*
A × D	6	444.164	2686.05**
T × A × D	18	865.537**	1145.38
Error	1287	443.585	(1392) 768.94

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 6. *Correlation coefficients estimated among days for arcsine (PropPc) in experiment 2, where observations based on ≤5 eggs were deleted and for number of eggs laid in each oviposition chamber (all data, including zero eggs). In each cell, coefficients are given for 3, 6 and 9 day old females, and overall*

Arcsine (PropPc)			
Day	2	3	4
1	−0.112	−0.133	0.187*
	0.155	0.115	0.249**
	0.045	−0.024	0.036
	0.055	0.003	0.154**
2		0.334**	0.085
		0.156	0.179
		0.231*	0.240*
		0.229**	0.167**
3			0.289**
			0.046
			0.370**
			0.217**
Egg number			
1	0.109	0.147	0.206*
	0.214*	0.404**	0.028
	0.358**	0.185*	0.319**
	0.233**	0.242**	0.191**
2		0.140	0.098
		0.307**	0.199*
		0.224*	0.339**
		0.252**	0.254**
3			−0.112
			−0.093
			−0.008
			−0.044

Experiment 4—Two-way and three-way choice for five yeast species

Of the 2880 females in the experiment, 512 (17.8 %) did not lay any eggs in the oviposition test period. As five yeast species were used, and in both two-way and three-way choice treatments, a number of different ANOVAs were done for the proportion of eggs laid on a particular yeast species, using results from the two-way choice treatments, the three-way choice treatments, and from both. The two-way versus three-way choice are referred to as treatments, and the various yeast species combinations as yeasts or yeast combinations. Treatment, block, population and yeasts were treated as fixed effects, and line within population as a random effect.

For all analyses, the number of eggs laid in each oviposition chamber was square root transformed,

Table 7. *Mean proportion of eggs (back-transformed) laid on the first named yeast in each of the 10 2-choice yeast combinations in experiment 4 (significance of differences by Scheffe's test indicated by lines), and the preference ranking of the five yeast species as average proportions of eggs on each yeast relative to the other four (back-transformed)*

Yeast species combination	Mean proportion	Ranked species	Mean proportion
Cs–Crc	0.804	Pc	0.717
Pc–Crc	0.795	Cs	0.534
Pb–Crc	0.777	Pb	0.528
Pc–Pb	0.759	Clo	0.439
Pc–Clo	0.658	Crc	0.282
Cs–Pb	0.537		
Clo–Crc	0.468		
Cs–Clo	0.424		
Clo–Pb	0.375		
Cs–Pc	0.352		

and the arcsine transformation was applied to the proportion of eggs laid on a particular yeast. All variances were homogeneous for the transformed data, but there were some cases of minor deviations from normality (noted below where appropriate).

Differences among all 20 yeast combinations for the number of eggs laid in each oviposition chamber were only marginally significant in ANOVA ($P = 0.03$), and Scheffe's test did not distinguish any as significantly different. The mean number of eggs per chamber (back-transformed) was 24.7, with a range over yeast combinations from 18.1 to 28.8.

ANOVA of all two-way choice treatments showed no significant differences among yeast combinations for egg number, although there were significant differences among populations ($P < 0.05$) and among lines within populations ($P < 0.001$). For the proportions of eggs laid on the first named yeast in each of the 10 yeast combinations, yeast combinations were highly significant ($P < 0.001$, see means in Table 7) and the line (population) × yeast combinations was significant ($P < 0.05$). Residuals in this analysis were not normally distributed ($W = 0.979$, $P < 0.001$).

The two-way choice yeast combinations were also analyzed for each yeast separately, i.e. for yeast i when paired with each of the other four yeast species. These ANOVAS (Table 8) of the proportions of eggs laid on yeast i showed significant differences among yeast combinations, except for Pc.

Table 8. *Analyses of variance, for each of the five yeasts separately (as yeast i) in the 2-choice yeast combinations, of the arcsine transformed proportions of eggs laid on yeast i, and back-transformed means for yeast combinations (significance of differences by Scheffe's test indicated by lines)*

Source	df	Mean squares				
Yeast *i*		Cs	Pc	Clo	Pb	Crc
Block	3	2695*	1349	1475	952	2387+
Population (P)	8	1910*	1406	1576	1992*	1931
Line/P	27	693	1347	2076*	691	2576**
Yeasts(Y)	3	15577***	2233+	5222**	23500***	7483***
P × Y	24	1339	1703*	1565	1174	879
Line/P × Y	81	1565**	988	1252	1246	1075
Error		(320)[a] 1009	(327) 1056	(331) 1044	(320) 1057	(307) 1000
W		0.980	0.965***	0.975**	0.976**	0.974**
Treatment means		Cs/Pc 0.352 Clo 0.424 Pb 0.537 Crc 0.804	Pc/Cs 0.648 Clo 0.658 Pb 0.759 Crc 0.795	Clo/Pc 0.342 Pb 0.375 Crc 0.468 Cs 0.576	Pb/Pc 0.241 Cs 0.463 Clo 0.625 Crc 0.777	Crc/Cs 0.196 Pc 0.205 Pb 0.223 Clo 0.532
Matrix	First yeast	Second yeast				
		Cs	Clo	Pb	Crc	
	Pc	0.648	0.658	0.759	0.795	
	Cs		0.424	0.537	0.804	
	Clo			0.375	0.468	
	Pb				0.777	

[a] Error df

+ $P<0.10$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 9. *Analyses of variance, for each of the two choice combinations in experiment 4, of the proportion of eggs (arcsine transformed) laid on the first named yeast species in each case*

Source	df	Mean squares			
		Cs/Pc	Cs/Clo	Cs/Pb	Cs/Crc
Block	3	852	2866*	1537	355
Population (P)	8	985	3063+	903	970
Lines/P	27	1192	1446+	1074	1730*
Error		(75)[a] 1216	(85) 904	(78) 1060	(73) 868
W		0.951**	0.969	0.982	0.965*
		Pc/Clo	Pc/Pb	Pc/Crc	
Block	3	350	912	1049	
Population (P)	8	1231	2614**	1571	
Lines/P	27	1200	584	1487+	
Error		(82) 1091	(81) 990	(80) 990	
W		0.953**	0.977	0.973	
		Clo/Pb	Clo/Crc	Pb/Crc	
Block	3	1262	2075	1113	
Population (P)	8	948	1023	1252	
Lines/P	27	1693+	1512	1187	
Error		(81) 1048	(74) 1069	(71) 1111	
W		0.972	0.968	0.975	

[a] Error df
+ $P<0.10$, * $P<0.05$, ** $P<0.01$

ANOVA of each two-way choice yeast combination (Table 9) was done to assess the expression of genetic variation for each combination. Significant differences among lines within populations were present only for Cs/Crc ($P < 0.05$), although three other combinations (Cs/Clo, Pc/Crc and Clo/Pb) were close to significance, $P = 0.054$, 0.084 and 0.052, respectively).

Each two-way choice yeast combination also was compared with the three three-way choice combinations that included the two-way yeasts, e.g., Cs/Pc with Cs/Pc/Clo, Cs/Pc/Pb and Cs/Pc/Crc. In this case, the variable analyzed was the proportion of eggs laid on Cs relative to Pc, i.e. for the three-way combinations—(number of eggs on Cs)/(number of eggs on Cs and Pc).

The yeast combinations effect in the ANOVA then tests whether the presence of any third yeast species affects the preference for the first named yeast in the two-way combination. These ANOVAs (Table 10) showed significant yeast combination effects in three of the 10 analyses, with two others near to significance ($P < 0.1$).

Each three-way yeast combination was analyzed separately for the proportion of eggs laid on each of the three yeasts, and for the proportions on yeast i versus yeast j (ignoring the third yeast). Thus six variables (all arcsine transformed) were subject to ANOVA for each of the 10 three-way combinations. Variation among lines within populations was that of primary interest, i.e. presence of genetic variation. In these 60 ANOVAs, lines within populations was significant for nine, and near to significance ($P < 0.10$) for another nine.

For the 10 three-way yeast combinations, the overall mean proportions (back-transformed) of eggs laid on each yeast were: Pc—0.306, Cs—0.260, Pb—0.251, Clo—0.240 and Crc—0.137. However, the relative preferences for each yeast commonly varied depending on which other two yeasts were present (Fig. 2). In all cases where Pc was included, it was the most preferred species. In the absence of Pc, Pb was most preferred. Cs was never the most preferred, while Clo was most preferred only in the Cs/Clo/Crc combination. Except for the Pc/Clo/Crc combination, Crc was always least preferred.

Experiment 5—Effects of mono- versus bicultures of yeast species

For the 10 two-way choice combinations, no eggs were laid in 168 of the 500 chambers (33.6%), while

Table 10. *Analyses of variance of the proportion of eggs (arcsine transformed) laid on yeast i relative to yeast j in each 2-choice yeast combination and in the three 3-choice combinations that included yeasts i and j (experiment 4)*

Source	df	Mean squares			
Yeasts i/j		Cs/Pc	Cs/Clo	Cs/Pb	Cs/Crc
Block	3	2353	5307**	2113	604
Population (P)	8	2966*	3618*	970	1717
Line/P	27	1303	1457	1427	2801*
Yeasts (Y)	3	225	714	3183*	653
P × Y	24	911	2656*	1115	834
Line/P × Y	80	1408	1355	1175	1504**
Error		(303) 1137	(282) 1156	(290) 1257	(255) 975
W		0.971***	0.971***	0.973***	0.958***
		Pc/Clo	Pc/Pb	Pc/Crc	
Block	3	1278	5502**	4611**	
Population (P)	8	1737	2030	2163	
Line/P	27	1729	1370	2398*	
Yeasts (Y)	3	2048	2238+	3444+	
P × Y	24	1119	1304	951	
Line/P × Y	80	1400+	952	1358	
Error		(284) 1121	(302) 1229	(291) 1128	
W		0.968***	0.969***	0.966***	
		Clo/Pb	Clo/Crc	Pb/Crc	
Block	3	543	3644*	4454**	
Population (P)	8	1518	2957	2239	
Line/P	27	2852+	2144+	2153***	
Yeasts (Y)	3	792	5932**	5092***	
P × Y	24	1147	1817	1652**	
Line/P × Y	80	1806**	1433	795	
Error		(270) 1147	(241) 1178	(265) 1154	
W		0.974**	0.971***	0.976*	

+ $P<0.10$, * $P<0.05$, ** $P<0.01$, ***$P<0.001$

none were laid in 379 of the 1000 mono- vs. biculture chambers (37.9%). In many of the chambers, preference for a particular yeast or yeast biculture was absolute, and residuals after arcsine transformation deviated from normality. Deletion of replicates with ≤ 5 eggs (32 for two-way choice, and 66 for mono- vs. bicultures) improved the fit (two-way choice—$W = 0.846$, $P < 0.001$), and mono- vs. bicultures—$W = 0.827$, $P < 0.001$), and these data were used in analyses. For egg number (all replicates, including 0 eggs), residuals deviated from normality (two-way choice—$W = 0.935$, $P < 0.001$, and mono- vs. bicultures—$W = 0.930$, $P < 0.001$), but the fit was not improved by square root transformation, and untransformed data were used in analyses.

In the two-way choice tests, differences among yeast combinations were significant for both egg number ($P < 0.01$) and for arcsine (PropY1), where PropY1 is the proportion of eggs laid on the first named yeast in each combination ($P < 0.001$). Mean egg numbers ranged from 11.5 for Clo/Crc to 33.3 for Pc/Crc, while proportions (back-transformed) ranged from 0.126 for Clo/Pb to 0.823 for Cs/Clo. The overall mean proportions (back-transformed) of eggs laid on each yeast were: Pb—0.671, Cs—0.612, Pc—0.570, Crc—0.472 and Clo—0.195.

For yeast mono- vs. bicultures, differences among yeast combinations again were significant for both egg number ($P < 0.001$) and for arcsine (PropM), where PropM is the proportion of eggs laid on the monoculture ($P < 0.01$). Mean egg numbers ranged from 9.6 for Clo vs. Clo + Cs to 36.4 for Crc vs. Crc + Pb, while mean proportions (back-transformed) ranged from 0.367 for Pb vs. Pb + Pc to

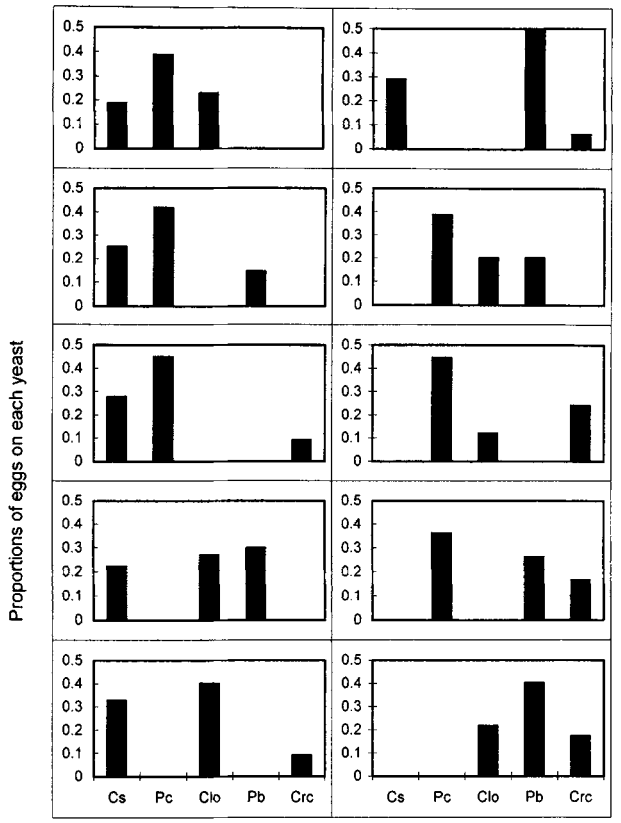

Fig. 2. Relative preferences for each yeast species in each of the 3-choice combinations.

0.921 for Cs vs. Cs + Clo. The overall mean proportions (back-transformed) of eggs laid on the monocultures were: Cs—0.784, Pc—0.727, Crc—0.724, Pb—0.646 and Clo—0.579.

The two-way choice and mono- vs. bicultures were compared for each pair of yeast species by ANOVA and orthogonal comparisons. For example, for Cs and Pc, the four combinations are (1) Cs and Pc, (2) (Cs + Pc) and Pc, (3) (Cs + Pc) and Cs, and (4) Pc and Cs, with comparisons made between (1) and (2), (2) and (3), and (3) and (4). Combinations were significantly different for six of the 10 yeast pairs (Table 11). In two cases, differences between the reciprocal mono- vs. bicultures were significant, while in three cases, there were significant differences between the two-way choice and corresponding mono- vs. biculture treatment. In all cases, however, the differences relate to an effect of the more preferred yeast species. For example, for Cs and Clo, Cs was the preferred yeast, so that a higher proportion of eggs were laid on Cs in combination (3)—(Cs + Clo) vs. Cs, but on the biculture in combination (2)—(Cs + Clo) vs. Clo.

Effects of biculture were further analyzed by taking for each pair of yeast species (say a and b), the mean arcsine (Prop a) for a vs. b, and the mean arcsine (Prop a) for a vs. (a + b), and computing the correlation coefficient between them for each of two subsets—(i) where a is preferred in the two-way choice, and (ii) where a is not preferred in the two-way choice. For the former, the correlation coefficient was positive ($r = 0.659$, $P < 0.05$) indicating that overall, where a particular yeast was preferred in the two-way monoculture choice, it was also preferred over the biculture. But for the latter, where a particular yeast was less preferred in the two-way monoculture choice, it was either less preferred, more preferred or equal relative to the biculture ($r = 0.384$, not significant).

Experiment 6—Heritability of oviposition preferences

The proportion of eggs laid on Pc was arcsine transformed, but all six variables still showed significant departure from normality (W values ranging from 0.863 to 0.952, all $P < 0.001$). All egg number vari-

Table 11. *Significant differences in back-transformed mean arcsine (PropY1) among combinations of 2-way choice and mono- vs. bicultures for pairs of yeast species in experiment 5, where PropY1 is the proportion of eggs laid on the first named yeast in each combination*

Combination[a]	Yeast species					
	Cs/Clo	Cs/Crc	Pc/Pb	Clo/Pb	Clo/Crc	Pb/Crc
1	0.823	0.713	0.656	0.126 *	0.136	0.821 **
2	0.666 *	0.456	0.790 ***	0.406	0.216	0.446
3	0.196	0.276	0.334	0.576 *	0.498 *	0.384
4	0.177	0.287	0.344	0.874	0.864	0.179
Significance Level in ANOVA	***	*	***	***	***	***

[a] Combination codes, e.g., using Cs/Clo: (1) Cs and Clo, (2) (Cs+Clo) and Clo, (3) (Cs+Clo) and Cs, (4) Clo and Cs
* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 12. *Analyses of variance of arcsine transformed proportions of eggs laid on Pc for day 1 (Pc1), day 2 (Pc2), day 3 (Pc3), days 1+2 (Pc12), days 2+3 (Pc23) and days 1+2+3 (Pc123) in the heritability analysis (experiment 6)*

Source	Pc1			Pc2			Pc3		
	df	MS	F	df	MS	F	df	MS	F
Block (B)	4	26139	27.33***	4	15751	20.01***	4	11709	12.59***
Sire/B	68	956	1.17	70	787	1.10	70	930	1.26
Dam/Sire	121	815	1.64***	143	714	1.38**	146	740	1.60***
Error	430	497		617	518		694	463	
	Pc12			Pc23			Pc123		
Block	4	24306	31.48***	4	15441	20.88***	4	19936	27.22***
Sire/B	70	772	1.34^{+}	70	739	1.40*	70	733	1.50*
Dam/Sire	144	578	1.34*	146	529	1.30*	146	487	1.32*
Error	658	433		758	407		768	370	

$^{+}$ $P<0.10$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$

ables were normalized by square root transformation. ANOVA and estimation of genetic parameters were done using the LSMLMW computer program, PC-2 version (HARVEY 1990), with sires nested within blocks, and dams within sires, and both sires and dams defined as random effects.

If the full design had been achieved (5 blocks × 15 sires × 3 dams/sire × 5 female offspring/dam), 1125 female records would have been available for analysis. However, fewer than 3 dams/sire produced progeny in some cases, and fewer than 5 female progeny/dam were available, or did not survive the three days of testing in other cases (some being lost at transfer), so that complete testing was done for 989 females. Substantial numbers of females did not lay any eggs on one or two of the test days, so that the numbers of females with records for the proportion of eggs laid on Pc were 624, 835 and 915 for days 1, 2 and 3, respectively. In order to maximize the data available for the estimation of heritability of the proportion of eggs laid on Pc, separate analyses were done for days 1, 2, 3, 1 + 2, 2 + 3, and 1 + 2 + 3. For egg number, however, zero eggs on any day was taken as a valid datum, so that a single analysis of variance and covariance gave the heritabilities and genetic and phenotypic correlations for the six variables. In order to obtain estimates of genetic and phenotypic correlations among the oviposition preference variables, and of these with egg numbers, ANOVA were done using only those females that laid eggs on both days 2 and 3 (771 females), and using those that laid on all three days (552 females).

ANOVA of the proportions of eggs laid on Pc (Table 12) showed that sire effects were significant ($P < 0.05$) only for Pc23 and Pc123 and approached this level of significance ($P < 0.10$) for Pc12, while dam effects were significant for each of the six variables. These ANOVA results are reflected in the heritability estimates (Table 13), where all paternal half-sib estimates (except for Pc123) are not significantly different from zero, while all maternal half-sib estimates and all full-sib estimates are significantly different from zero.

For egg number, both sire and dam effects were significant for all six variables (Table 14), and paternal half-sib estimates of heritabilities and genetic correlations, and estimated phenotypic correlations are given in Table 15. All heritability estimates are significantly different from zero. Genetic correlations between egg numbers on consecutive days (egg 1–egg 2 and egg 2–egg 3) are much higher than the correlation for day 1–day 3, and all genetic correlations are greater than the respective phenotypic correlations. The repeatability of egg number over the three days ranged from 0.342 to 0.367.

ANOVA of data for those females that laid eggs on both days 2 and 3, and for those that laid on all of days 1, 2 and 3 gave results for the proportion of eggs laid on Pc similar to those in Table 12, i.e. dam effects were significant, but sire effects generally were not significant. Paternal half-sib estimates of heritability were of similar magnitude to those in Table 13, but none differed significantly from zero. Maternal half-sib and full-sib heritability estimates were generally higher than those in Table 13, but only three of the nine comparisons were significant ($P < 0.05$).

For egg number in these analyses, the sire variance component was negative for three of the nine vari-

ables, and for the other six, the paternal half-sib heritability estimate was not significantly different from zero. These contrast with the significant paternal half-sib estimates in Table 15, derived from data using all females, including those that laid no eggs on one or two of the three days. However, maternal half-sib and full-sib estimates from the three analyses are not significantly different (Table 16 gives the full-sib estimates).

Because of the negative sire variance components, many paternal half-sib genetic correlations were not estimable. Thus genetic correlations among PropPc variables and between these and egg number variables were estimated from full-sib variance and covariance components. Estimates made from the days 2 and 3 data were not significantly different from those from days 1, 2 and 3 data, and only the latter are given (Table 17). Further, none of the genetic correlations between any PropPc and egg number were significantly different from zero, and all corresponding phenotypic correlations were close to zero (not shown).

Experiment 7—Generation means analysis

The least squares means for all planned comparisons are given in Table 18, with significant comparisons indicated both for the least squares means testing and for the log-linear modelling of the actual proportions of eggs laid on Pc. For all four pairs of lines, the high and low preference parent lines were significantly different, as expected, with general concordance between least squares means and log-linear testing. In other comparisons, these statistical tests were not always concordant, so that indicated significant differences by either test should be interpreted cautiously, e.g., the apparent extranuclear (maternal or cytoplasmic) effect for lines T9 and T3. All comparisons testing for X chromosome effects were non-significant, but significant autosomal effects were indicated, except for lines IG2 and T3. For lines T9 and T3, and for IG2 and MB6, some of these significant effects show differences opposite to that expected. However, given the absence of X chromosome effects, genotypes with different X chromosome constitution but the same percentage of autosomal genes can be pooled—increasing replicate numbers and the sensitivity of analysis.

The different genotypes thus were reduced to six, viz. two parental, F_1 (genotypes 3 and 4, Table 1), F_2 (5 and 6), back-crosses to the high parent (7, 8, 11 and 12) and back-crosses to the low parent (9, 10, 13 and 14). Distributions of the proportion of eggs laid on Pc for each of these genotypes and in each line combination (Fig. 3) emphasise the quantitative nature of the observed oviposition preferences—all 24 genotypes showing proportions ranging from zero to unity. While both the high preference lines (T9 and IG2) have the highest frequency of replicates in the range 0.9–1.0, the distributions for the two low preference lines differed. For T3, the mode is around 0.4, but MB6 is bimodal. Comparisons of the least squares means for each of these six genotypes for each pair of lines (Table 19) provide strong support for autosomal genes affecting oviposition preferences for the two yeast species. However, these effects are not the same for all four line combinations, and overall, are not simply additive. Expected means for the crosses, assuming additivity (Table 19) show that where T9 is the high preference line, the F_1 mean is much less than expected, being approximately equal to or less than the low line mean. Further, observed means for the F_2 and the back-crosses are all less than expected. Autosomal genes for high preference for Pc in line T9 clearly are recessive to genes for low preference in both T3 and MB6, with even possible overdominance effects in the T9/T3 line combination. However, these dominant effects for low preferences in T3 and MB6 are not apparent in relation to the high preference line IG2. Genetic effects are close to additive for IG2/T3, while for IG2/MB6, the F_1 observed mean is close to that expected for additivity, but both back-crosses deviate. The observed mean for the back-cross to the high line (IG2) is less than expected, but the back-cross to the low line (MB6) is

Table 13. *Mean proportions of eggs laid on Pc (back transformed least squares means and confidence intervals), and paternal half-sib, maternal half-sib and full-sib estimates of heritability (standard errors in parentheses)*

Variable	N	Mean	Heritability (SE)		
			Paternal half-sib	Maternal half-sib	Full-sib
Pc1	624	0.926(0.897–0.950)	0.090(0.108)	0.664(0.175)	0.377(0.087)
Pc2	835	0.939(0.922–0.955)	0.039(0.075)	0.361(0.134)	0.200(0.063)
Pc3	915	0.955(0.939–0.969)	0.105(0.078)	0.500(0.129)	0.302(0.066)
Pc12	877	0.923(0.904–0.940)	0.132(0.085)	0.304(0.126)	0.218(0.062)
Pc23	979	0.937(0.921–0.951)	0.141(0.079)	0.248(0.112)	0.194(0.056)
Pc123	989	0.925(0.909–0.940)	0.177(0.083)	0.256(0.111)	0.217(0.057)

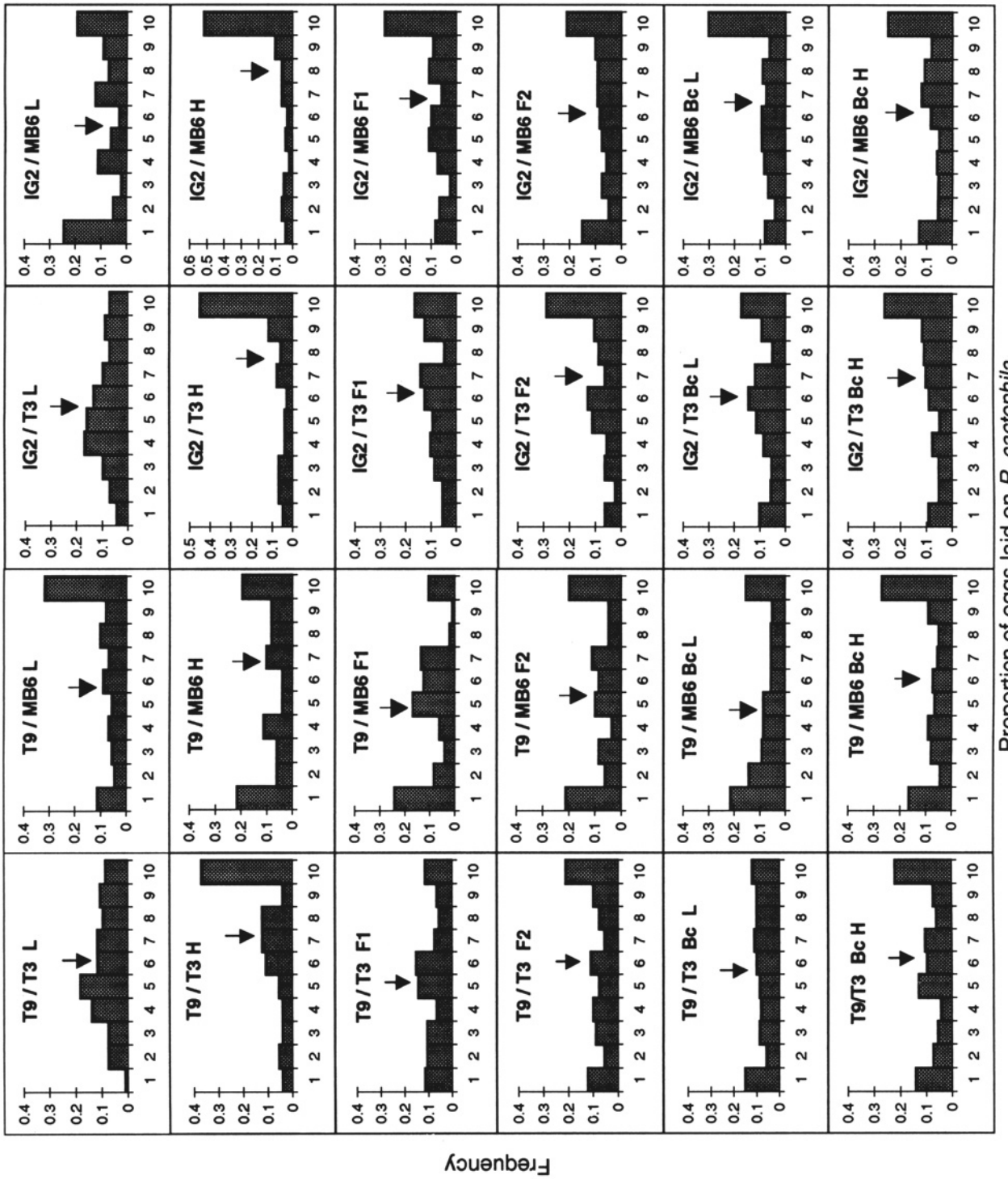

Fig. 3. Frequency distributions for the proportion of eggs laid on *P. cactophila* for parental, F_1, F_2 and backcross genotypes of each of four combinations of high (T9 and IG2) and low (T3 and MB6) Pc preference lines, with the mean proportion for each indicated by the arrow. Note the different y-axis scale for IG2/T3 H and IG2/MB6 H.

Table 14. *Analyses of variance of egg number (square root transformed) on day 1, day 2, day 3, days 1 and 2, days 2 and 3, and days 1, 2 and 3 in the heritability analysis (experiment 6)*

Source of variation	df	Mean squares					
		Egg1	Egg2	Egg3	Egg12	Egg23	Egg123
Blocks (B)	4	645.38***	699.37***	308.97**	1315.79***	903.18***	1449.16***
Sires/B	70	21.91***	14.79**	10.97**	29.15***	19.36***	30.60***
Dams/Sire	146	9.90***	8.62***	6.51**	13.64***	10.17***	14.54***
Error	768	6.27	5.63	4.79	7.73	6.15	7.76

** $P<0.01$, *** $P<0.001$

greater than expected, and the means for both back-crosses are essentially the same as the F_1 mean. This would imply some kind of epistasis in the F_1 which is dominant to the genotypes of both parental lines. The bimodal distribution of preferences for MB6 (Fig. 3) suggests that this line is polymorphic for genes affecting preferences, and some of these genes interact with those in IG2 in a different way from any genetic effects in the T9/MB6 crosses.

Joint scaling tests—genotype means. For all four line combinations, no sex-linked or maternal effects were significant. Thus the observed genotype means for the reduced set of six genotypes (both parents, F_1, F_2, and both back-crosses) were fitted to the model incorporating the six parameters—mean, additive, dominance, additive × additive, additive × dominance and dominance × dominance. With six genotypes and six parameters estimated, there are no degrees of freedom to test the fit of the model, so that the estimated standard error of each parameter was used to test if the parameter was significantly different from zero. For the line combination IG2/MB6, the additive × dominance epistatic effect ($\hat{j}$) was significant ($t = 5.741$, $P < 0.001$), but no digenic epistatic effects were significant for any of the other three line combinations. In order to test for higher order genic interaction and/or linkage (MATHER and JINKS 1982, Ch. 5), the non-significant digenic epistasis terms were dropped from the model ($\hat{i}$ and $\hat{j}$ for IG2/MB6, all three for the other line combinations). All four resulting chi-square tests were non-significant, so that higher order epistasis and/or linkage do not contribute significantly to the differences in oviposition preferences in any of the four line combinations. For T9/T3 and T9/MB6, this model incorporating the mean and additive and dominance effects was thus the best (Table 20), but for IG2/T3 and IG2/MB6, the dominance parameter was not significant. It was therefore dropped from the models for these two line combinations, to give their final models (Table 20). These results for the joint scaling tests both validate and quantify the interpretations above that were based on comparisons of least squares means. For all four line combinations, estimates of the number of effective factors (Table 20) are not significantly different from zero.

ANOVA of the number of eggs laid by each female in the preference testing (after deleting females that laid no eggs, and square root transformation) showed highly significant differences among genotypes ($P < 0.001$) for all four line combinations.

Joint scaling tests of the genotype means for egg number were done by fitting three models—(i) additive, dominance and sex-linked effects, (ii) additive, dominance and maternal effects, and (iii) additive, dominance and digenic epistasis. From these, the best fitting model (Table 21) was derived. Sex-linked and additive × dominance epistasis were not significant for any of the four line combinations, but all four differed in the contributions from other effects—in presence or absence, magnitude or sign. As might be expected, and in agreement with the heritability estimates (Experiment 6), non-additive and/or maternal effects predominate.

Experiment 8—Selection for oviposition preferences

The preliminary test of the 27 isofemale lines showed highly significant variation among lines ($P < 0.001$) for arcsine transformed proportion of eggs laid on Pc. Back-transformed means for the lines ranged from 0.084 to 0.892, with a mean over all lines of 0.582. The estimated isofemale heritabilities were 0.063 when all females were included, and 0.102 when females laying ≤ 5 eggs were deleted from the analysis, leading to heritabilities of individual differences in oviposition preference of 0.130 and 0.215, respectively.

Selection for increased and decreased preference for oviposition on Pc (Fig. 4) showed significant separation by generations 4 and 5 of Pc^+ and Crc^+ lines that were from the same initial base. For all lines, the proportion of eggs laid on Pc increased in generation 1, and then decreased in generation 2, before selection responses started to become appar-

Table 15. *Heritabilities and genetic correlations (paternal half-sib estimates–sire components of variance and covariance), and phenotypic correlations for egg number (square root transformed) on day 1, day 2, day 3, days 1 and 2, days 2 and 3, and days 1, 2 and 3. Standard errors (in parentheses) are minimum estimates. Heritabilities are on the diagonal, genetic correlations above, and phenotypic correlations below*

	Egg1	Egg2	Egg3	Egg12	Egg23	Egg123
Egg1	0.453(0.119)	0.755(0.134)	0.550(0.171)	0.933(0.038)	0.670(0.128)	0.862(0.059)
Egg2	0.367	0.273(0.097)	0.925(0.144)	0.939(0.040)	0.992(0.032)	0.990(0.042)
Egg3	0.367	0.342	0.243(0.093)	0.791(0.130)	0.973(0.046)	0.881(0.071)
Egg12	0.783	0.844	0.409	0.456(0.120)	0.890(0.054)	0.989(0.013)
Egg23	0.439	0.828	0.788	0.770	0.356(0.107)	0.955(0.022)
Egg123	0.739	0.763	0.732	0.908	0.920	0.459(0.120)

ent. Although all experimental procedures were the same throughout the experiment, including the one batch of autoclaved *O. stricta* used for all medium preparation, environmental effects presumably outweighed genetic responses in these two generations.

The realized heritabilities of index values were estimated from regression of response (generation means) on cumulative selection differential for generation 1 (start of index selection) to generation 5. For the Pc^+ lines, the Crc^+ lines and for divergence between Pc^+ and Crc^+, variances, slopes and intercepts were not significantly different among replicates, so that pooled estimates were computed: 0.133 ± 0.115 (not significant) for Pc^+, 0.425 ± 0.093 ($P < 0.001$) for Crc^+, and 0.297 ± 0.067 ($P < 0.01$) for divergence. That is, no genetic variation was detectable when selecting for increased Pc preference (Pc^+), but highly significant variation when selecting against Pc preference (Crc^+).

Experiment 9—Adult performance on different yeasts, and comparison of larval and adult preferences

For ANOVA, larvae–adult viability was arcsine transformed, average body weights transformed to $1/\sqrt{wt}$ and both measures of fecundity transformed to $\log_e(\text{eggs} + 1)$. Lines were significantly different for developmental time, viability, average female weight and both measures of fecundity ($P < 0.001$ for all). However, line means (Table 22) show that for developmental time and viability, the high Pc preference lines (IG2 and T9) were not consistently superior or inferior to the low Pc preference lines (MB6 and T3). In general, lines with higher viability (IG2 and MB6) had longer developmental times and higher average weights. For average female body weight, however, the low Pc preference lines were significantly ($P < 0.05$) heavier than the high preference lines (back-transformed means of 0.263 and 0.248 mg, respectively). The high Pc preference lines showed higher fecundity (both measures) than the low preference lines.

Yeast effects were significant ($P < 0.001$) for developmental time (shorter on Pc - 14.9 days vs. 16.1 days on Crc) and for fecundity (higher on Pc - 2.12 vs. 0.86 on Crc for eggs/day of female life, and 1.78 vs. 0.70, respectively for eggs/test day). A significant line × yeast interaction for viability was due to T9 having lower viability on Pc than on Crc, whereas all other lines had lower viability on Crc.

All lines had higher fecundity (both measures) on Pc, but the difference in egg numbers for Pc vs. Crc was much greater for the high preference lines than for the low preference lines (significant line × yeast interaction—$P < 0.05$). Comparing high vs. low lines, back-transformed means for eggs/day of female life were 3.36 and 0.95 for the high lines on Pc and Crc, respectively, and 1.22 and 0.78 for the low lines. For eggs/test day, these means were 2.62 and 0.73, and 1.13 and 0.68. For fecundity, the high Pc preference lines clearly perform relatively better on Pc as compared with Crc than do the low preference lines.

Larvae (0–2 h old) of all four lines showed high attraction to Pc (mean over lines and times (excluding 5 min observation) – back-transformed = 96.6%), but lines were not significantly different ($P < 0.10$). The two high Pc preference lines (IG2 and T9) had the highest and lowest attraction respectively to Pc.

Table 16. *Full-sib estimates of heritability of egg number variables from the full data set (including 0 eggs), and from data for those females that laid eggs on both days 2 and 3, and on all days 1, 2 and 3 (standard errors in parentheses)*

	Full data set	Days 2 and 3	Days 1, 2 and 3
Egg1	0.432(0.070)	–	0.276(0.089)
Egg2	0.336(0.065)	0.289(0.073)	0.236(0.087)
Egg3	0.262(0.060)	0.297(0.073)	0.373(0.094)
Egg12	0.489(0.073)	–	0.404(0.095)
Egg23	0.413(0.069)	0.398(0.078)	0.387(0.095)
Egg123	0.521(0.074)	–	0.460(0.097)

Table 17. *Genetic (above diagonal) and phenotypic correlations (below diagonal) among the six measures of proportion of eggs laid on Pc, estimated from the data for those females that laid eggs on all three test days (standard errors of genetic correlations in parentheses)*

	Pc1	Pc2	Pc3	Pc12	Pc23	Pc123
Pc1		0.517(0.194)	0.344(0.194)	0.927(0.062)	0.544(0.186)	0.839(0.092)
Pc2	0.242		0.348(0.217)	0.758(0.101)	0.826(0.096)	0.749(0.119)
Pc3	0.260	0.167		0.347(0.196)	0.737(0.113)	0.657(0.132)
Pc12	0.729	0.738	0.279		0.649(0.132)	0.905(0.043)
Pc23	0.321	0.724	0.706	0.696		0.842(0.066)
Pc123	0.656	0.642	0.624	0.890	0.879	

In the separate experiment testing third instar larvae, however, line effects were highly significant at the 30 min observation ($P < 0.01$), although not significant at 15 min. The mean (back-transformed) proportion of larvae on Pc at 30 min were: IG2 = 0.812, T9 = 0.894, MB6 = 0.609 and T3 = 0.732. Thus the high Pc female oviposition preference lines (IG2 and T9) also show higher Pc preference as third instar larvae.

DISCUSSION

We have demonstrated that OSP of *D. buzzatii* females is heritable, with evidence from isofemale lines, direct estimation of heritability, generation means analysis and short term selection. Further, this genetic variation in OSP relates to components of their natural habitats, namely, the yeast species that occur in cactus rots.

Nevertheless, OSP is clearly a labile trait, one subject to environmental variation that will dilute the genotype-specific effects. In previous experiments (BARKER 1992), specificity of OSP (proportion of eggs laid on the preferred yeast) was reduced as the number of eggs laid increased, indicating some degree of interference or required spacing between females at oviposition. Any such interference or crowding effect at the preferred oviposition site may be confounded with an egg load effect. That is, females carrying a mature egg and motivated to lay will be more likely to oviposit on a less preferred yeast if they are disturbed at or fail to gain access to the preferred yeast because of crowding. The significant effects of density (number of females per replicate oviposition plate) in experiment 1 (Tables 3 and 4) clearly indicate such interference/egg load phenomena in females, with additional density effects due to male–female interactions. Further, OSP specificity decreased as the time allowed for oviposition was increased. Conceivably, OSP may change as a female ages, but as there were no significant effects of age on PropPc in experiment 2 (Table 5), this is most likely due to an effect of egg density on the oviposition surface.

When the same females were tested over consecutive days (experiments 2, 3 and 6), there were no significant differences among days in experiments 3 and 6, but the proportion of eggs laid on Pc was significantly higher on day 1 in experiment 2. The conditioning treatment × age × day interaction was the only other significant effect in experiment 2 (Table 5). No clear pattern could be discerned for this interaction, but the higher overall PropPc on day 1 was due primarily to 3-day old females. As the females in experiments 3 and 6 were 9 or 10 days old when first tested, young females may have an initially higher preference for Pc, or alternatively, their lower fecundity may have obviated density/interference effects. In any case, the significant overall correlations among days 2, 3 and 4 for PropPc, but not for day 1 with day 2 or day 3 (Table 6), show that initial responses on first exposure to yeast choice are in some way different.

In addition to the significant effects of the imposed treatments in experiments 1–3, uncontrolled environmental factors and possible variation in the physiology of the flies have a major influence on expression of OSP. Even though all experimental procedures were standardized, the repeatability over consecutive days of testing the same females in experiments 2 and 3 was only 0.138 and 0.123, respectively.

Oviposition site preference is one component of the complex of behaviours exhibited by females seeking a place to oviposit. In turn, this may be part of, or interact with, broader processes of resource use or habitat preference (HOFFMANN 1985; JAENIKE 1986; PAPAJ and RAUSHER 1983). These components have been referred to in various ways in different systems that have been studied. In polyphagous insect species (particularly butterflies), host rank order preference and specificity (number of acceptable hosts) are defined as separate components (SINGER 1982; THOMPSON 1988b). LOFDAHL (1986) and COURTNEY and CHEN (1988) studied genetic variation for habitat

Table 18. *Least squares means of arcsine transformed proportion of eggs laid on P. cactophila for each genotype in each of the planned comparisons (genotype with expected higher preference given first). In addition to tests of the difference between these means for each comparison, comparisons that were significant in log-linear models also are indicated. Comparisons significant by either method that are opposite to expectation are underlined (experiment 7)*

Effects tested	Genotype comparison	Least squares means (SE) for each genotype in each comparison							
		Lines T9/T3		Lines T9/MB6		Lines IG2/T3		Lines IG2/MB6	
Parents	1–2	58.3(2.6)	49.0(2.2)**,A	52.9(2.5)	40.5(2.4)***,B	61.1(2.1)	45.4(2.0)***,C	65.6(2.4)	40.6(2.3)***,C
Maternal	4–3	46.9(3.0)	36.7(3.0)*	41.1(3.3)	35.8(3.7)	50.5(2.8)	51.2(2.7)	49.1(3.0)	56.5(3.1)
X chromosome	6–5	51.3(3.3)	46.0(3.2)	40.6(4.0)	41.5(3.6)	53.8(2.8)	51.8(2.7)	49.4(3.1)	46.5(3.0)
	8–7	46.9(3.5)	49.5(3.3)	45.6(3.7)	49.6(3.6)	57.7(2.8)	52.4(2.8)	50.2(3.6)	44.4(3.1)
	10–9	46.6(3.1)	46.3(3.2)	36.5(4.4)	38.9(3.4)	50.4(2.7)	48.6(2.7)	53.4(3.0)	52.5(3.0)
Autosomes	1–8	58.3(2.6)	46.9(3.5)*	52.9(2.5)	45.6(3.7)	61.1(2.1)	57.7(2.8)	65.6(2.4)	50.2(3.6)***,C
	9–2	46.3(3.2)	49.0(2.2)	38.9(3.4)	40.5(2.4)	48.6(2.7)	45.4(2.0)	52.5(3.0)	40.6(2.3)**,A
	7–3	49.5(3.3)	36.7(3.0)**	49.6(3.6)	35.8(3.7)**,A	52.4(2.8)	51.2(2.7)	44.4(3.1)	56.5(3.1)**,A
	7–4	49.5(3.3)	46.9(3.0)	49.6(3.6)	41.1(3.3)A	52.4(2.8)	50.5(2.8)	44.4(3.1)	49.1(3.0)
	3–10	36.7(3.0)	46.6(3.1)*,A	35.8(3.7)	36.5(4.4)	51.2(2.7)	50.4(2.7)	56.5(3.1)	53.4(3.0)
	4–10	46.9(3.0)	46.6(3.1)	41.1(3.3)	36.5(4.4)A	50.5(2.8)	50.4(2.7)	49.1(3.0)	53.4(3.0)
	5–13	46.0(3.2)	43.2(3.1)	41.5(3.6)	36.3(3.2)	51.8(2.7)	46.7(2.7)	46.5(3.0)	49.9(3.2)
	5–14	46.0(3.2)	41.3(3.1)	41.5(3.6)	43.6(3.4)	51.8(2.7)	46.6(2.8)	46.5(3.0)	61.9(3.3)***
	11–6	43.9(3.3)	51.3(3.3)B	41.9(4.0)	40.6(4.0)	53.3(2.7)	53.8(2.8)	55.6(3.0)	49.4(3.1)
	12–6	52.3(3.6)	51.3(3.3)	45.9(3.4)	40.6(4.0)	50.9(2.9)	53.8(2.8)	51.7(2.9)	49.4(3.1)
	7–10	49.5(3.3)	46.6(3.1)	49.6(3.6)	36.5(4.4)*	52.4(2.8)	50.4(2.7)	44.4(3.1)	53.4(3.0)*

Significance of differences between least squares means—* $P<0.05$, ** $P<0.01$, *** $P<0.001$
Significant comparison in log-linear model—A $P<0.05$, B $P<0.01$, C $P<0.001$

selection using a novel host, with estimation of two components (i) host acceptance, and (ii) number of eggs laid if initial acceptance occurred. In this case, the individuals tested have no choice of hosts, so that the experiments relate not to genetic variation for habitat choice, but to genetic variation for host shifts.

Where a choice is available to females seeking oviposition sites, JAENIKE (1986) has defined two components, (i) settling behaviour—attraction from a distance and settling at a potential breeding site, and (ii) oviposition site preference—acceptance or rejection of this site for oviposition. Genetic variation for both these components was found by JAENIKE (1986) for *D. tripunctata*, but his results indicated them to be genetically independent.

For *D. buzzatii*, these two components are relevant at the levels of between rots and within rots. In these rots, the resource used by the flies is the microbial community of yeasts and bacteria that provide food for both adults and larvae. The major stimulus to flies seeking a suitable feeding and/or breeding site is undoubtedly olfactory, and volatiles produced by the microbial community most likely provide the cue (FOGLEMAN 1982). No data are available for the bacterial communities of cactus rots. However, natural rots contain 1–5 yeast species with an average of about 2–3, but there is significant heterogeneity among rots in yeast species composition. From one locality in the Hunter Valley, NSW, and from several localities in Queensland, 944 yeast isolates were identified. For the Hunter Valley locality, there were significant differences in the yeast community among types of rot and among seasons (BARKER et al. 1983). For the total yeast data, temperature, pH and age of the rot all influenced yeast community composition (BARKER et al. 1984). Further, significant differences among rots sampled at the same time in one locality were found for yeast species abundance and fre-

Table 19. *Least squares means of arcsine transformed proportion of eggs laid on P. cactophila for parent lines and crosses, significance of differences among them (comparisons of least squares means, and from log-linear models), and expected means for the crosses, assuming additivity (experiment 7)*

Genotype	Least squares mean	Expected mean	Genotype				
			BC(H)	F_1	F_2	BC(L)	L
Lines T9/T3							
H	58.3	51.3	**,A	***,C	**,A	***,B	**,A
BC(H)	48.2	49.4		*			B
F_1	41.8	47.6			*		*,A
F_2	48.6	47.6					A
BC(L)	44.4	45.7					
L	49.0	43.8					
Lines T9/MB6							
H	52.9	49.1	*	***,C	**,A	***,C	***,B
BC(H)	45.8	46.0		*,C		**,B	
F_1	38.3	42.9				B	A
F_2	41.4	42.9					
BC(L)	38.9	39.7					
L	40.5	36.6					
Lines IG2/T3							
H	61.1	58.6	**,A	***,C	**	***,C	***,C
BC(H)	53.7	55.2				**,B	***,C
F_1	50.8	51.7					
F_2	52.9	51.7				*	**,B
BC(L)	48.1	48.2					A
L	45.4	44.7					
Lines IG2/MB6							
H	65.6	58.5	***,C	***,B	***,C	***,C	***,C
BC(H)	50.6	55.2					***
F_1	52.3	51.9					***,B
F_2	48.3	51.9				*	*
BC(L)	54.2	48.7					***,A
L	40.6	45.4					

Significance of differences between least squares means—* $P<0.05$, ** $P<0.01$, *** $P<0.001$
Significant comparison in log-linear model—$^{A}P<0.05$, $^{B}P<0.01$, $^{C}P<0.001$

Table 20. *Estimates (± SE) of effects contributing to the differences between parental isofemale lines in oviposition preferences for each of four combinations of high (T9 and IG2) and low (T3 and MB6) P. cactophila preference lines, and number of effective factors (ne) for each line combination (SE in parentheses)*

Effect	Line combinations			
	T9/T3	T9/MB6	IG2/T3	IG2/MB6
m	53.151 ± 1.500	46.584 ± 1.541	51.662 ± 0.708	52.004 ± 0.799
d	4.325 ± 1.391	6.435 ± 1.425	6.987 ± 1.187	12.451 ± 1.703
h	−12.056 ± 2.707	−8.549 ± 2.901		
j				−32.140 ± 5.609
Chi-square	1.172	0.186	3.036	3.469
Df	3	3	4	3
ne	−3.00 (74.5)	1.54 (12.3)	5.34 (64.6)	7.74 (64.6)

quency (BARKER et al. 1987). Thus choice among rots (settling behaviour) likely depends on long distance attraction and responses to specific combinations of yeast and possibly bacterial species.

Evidence for differential long distance attraction to yeast species has been found by ARMSTRONG (1992), using a wind tunnel olfactometer (HOFFMANN et al. 1984) in the laboratory. This apparatus tests the in-flight response to attractant sources in the presence of light, thus simulating long distance attraction in nature. Differences between yeast species were significant in five of the six pairwise tests possible with four yeast species (*Candida sonorensis*, *Pichia cactophila*, *Clavispora opuntiae* and *Cryptococcus cereanus*). In further tests with these four species, comparing the attractiveness of pairs of yeast monocultures with each one of the two paired yeasts (12 tests), the pair of yeasts was significantly more attractive than the single yeast in seven tests.

Once females respond to long distance cues and settle on or in a rot, they may or may not oviposit, and if the rot substrate were a homogeneous mix of microorganisms, no other choice would be possible. However, within rots, the yeast species distribution is patchy, with multiple samples taken at the one time showing diversity in yeast species abundance and frequency (BARKER et al. 1987), so that there is the potential for females to exhibit oviposition site preference for different yeast species. It is this level of choice between yeast species that is the focus of the experiments reported here.

Theoretical models of genotype-specific habitat selection (TEMPLETON and ROTHMAN 1981; RAUSHER 1984; GARCIA-DORADO 1986; GARCIA-DORADO 1987; HEDRICK 1990a) have shown that the conditions for the maintenance of polymorphism are substantially broader than for the LEVENE (1953) model of soft selection in a heterogeneous environment. These models assume a single locus that pleiotropically influences both habitat selection and fitness (generally expressed as viability only). Further, random mating over the whole population is assumed, and the young individuals of both sexes choose a habitat where selection occurs, or female parents select habitats and their progeny undergo selection within these habitats. Even when viability selection is weak or absent, a stable polymorphism can still be maintained (RAUSHER 1984; HEDRICK 1990a). Other models (HEDRICK 1990b; DE MEEÛS et al. 1993) assume two loci, one influencing habitat selection and the other relative viability. In the model by HEDRICK (1990b), the loci act independently, but effects of linkage are considered. DE MEEÛS et al. (1993) assume an epistatic effect of the adaptive locus on the habitat selection locus, but no linkage, and mating within each habitat. With epistatically determined habitat selection (DE MEEÛS et al. 1993) and soft selection, polymorphism is maintained at both loci. However, if the loci act independently (HEDRICK 1990b), the probability of a stable polymorphism at the viability locus, particularly for weak selection at this locus, is greatly increased only for tight linkage. In still other models where selection by females determines the habitat in which their progeny will develop, RAUSHER (1984) and DIEHL and BUSH (1989) have shown that polymorphism can be maintained at the locus affecting habitat preference, even with random mating and without the locus affecting fitness in the 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.

In all these models, genotype-specific habitat selection acts to maintain polymorphism at the locus

affecting habitat selection, and in some cases, may also maintain polymorphism at other loci determining fitness in the different habitats. What then is the relevance of these models to the genetic variation shown here for OSP for yeast species in *D. buzzatii*, and to the consequent maintenance of genetic variation at other loci?

Firstly, all models considered assume density-dependent (soft) selection, acting independently in each habitat. Density-dependent mortality in natural populations of *D. buzzatii* has been found by QUEZADA-DÍAZ et al. (1997) for flies breeding in rotting cactus fruit, and was inferred by PROUT and BARKER (1989) to be occurring in cladode rots. These findings relate to mortality at the level of individual rots. However, larvae of the cactophilic *D. mojavensis* selectively feed on the yeasts within natural rots (FOGLEMAN et al. 1981, 1982), *D. buzzatii* larvae show differential preferences for yeast species in laboratory experiments (BARKER et al. 1988), and third instar larvae of lines differing in OSP for the yeasts Pc and Crc show concurrent differences in preferences for these two yeasts (experiment 9). Thus females with a genotype conferring OSP for a particular yeast produce larvae that selectively feed on that yeast, potentially leading to density-dependent selection among those larvae preferentially feeding on each yeast species.

Secondly, some models assume a positive association of habitat selection and fitness, i.e. that individuals (or females) choose the habitat in which they (or their progeny) are most fit. For *D. buzzatii*, there is some evidence for such a positive association between genotype-specific OSP and progeny fitness. At the species level, there is general correspondence between yeasts preferred by females for oviposition, yeasts preferred by second instar larvae, and pre-adult fitness (viability and developmental time) when raised on different yeasts (VACEK 1982). Using four isofemale lines and four pairs of yeast species, ARMSTRONG (1992) found a weak correspondence between yeast of oviposition choice and pre-adult performance (viability, developmental time and body weight of emerging adults), but these lines did not show extreme diversity for OSP. Specifically for the Pc/Crc yeast choice, however, two of the lines showed higher OSP for Pc, and two higher OSP for Crc. Progeny of three of these lines, when raised on cactus medium seeded with either Pc or Crc, had significantly higher performance on the yeast preferred for oviposition by their female parents. Differences among the four lines with contrasting Pc and Crc OSP (experiment 9) in pre-adult viability and female body weight when raised on media seeded with either Pc or Crc were not positively associated with OSP (Table 22), but a positive association was found for fecundity. The high Pc preference lines had higher average fecundity than the low Pc preference lines (Table 22), specifically due to their higher fecundity when raised on and then feeding on Pc. This positive association, presumably genetic and due to pleiotropy or linkage disequilibrium, will for most theoretical models increase the probability of maintenance of variation at both OSP and fitness loci (if they are different loci).

Thirdly, while most models assume random mating preceding habitat choice, local mating (random mating within each habitat) will broaden the conditions for polymorphism. That is, if males and females of the same genotype choose the same habitat and then mate, mating will be essentially assortative at the population level for preference alleles. Alternatively, an assortative mating mechanism that does not require the build-up of different assortative mating alleles in different habitats would have the same

Table 21. *Estimates (± SE) of effects contributing to the differences between parental isofemale lines in numbers of eggs laid for each of four combinations of high (T9 and IG2) and low (T3 and MB6) P. cactophila preference lines*

Effect	Line combinations			
	T9/T3	T9/MB6	IG2/T3	IG2/MB6
m	3.302 ± 0.877	9.059 ± 0.842	4.513 ± 0.095	6.590 ± 0.150
d	0.154 ± 0.119	−0.949 ± 0.112	−0.889 ± 0.169	−2.116 ± 0.199
h	6.918 ± 1.999	−3.628 ± 1.912		−0.563 ± 0.261
i	2.635 ± 0.864	−1.799 ± 0.830	0.771 ± 0.179	
l	−3.444 ± 1.205	2.615 ± 1.148		
dm			0.352 ± 0.130	0.376 ± 0.150
hm				−0.334 ± 0.161
Chi-square	0.124	0.226	5.612	5.671
Df	1	1	5	4

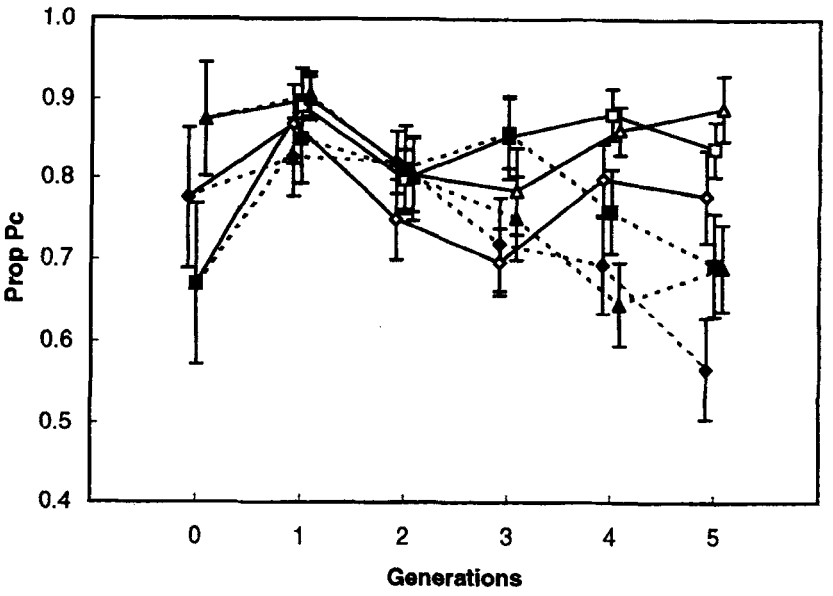

Fig. 4. Responses to selection for increased oviposition preference for Pc (Pc^+: solid lines—◇ replicate 1, □ replicate 2, △ replicate 3) and decreased preference for Pc (Crc^+: broken lines—◆ replicate 1, ■ replicate 2, ▲ replicate 3).

effect of facilitating maintenance of polymorphism. One such mechanism is conditioning or early adult experience, where prior exposure to a particular resource enhances a female's tendency to oviposit on that resource (JAENIKE 1988). While there is no evidence of any effect of early adult experience in *D. buzzatii* (HEDRICK et al. 1990; experiment 2), local assortative mating is a possibility. In field experiments on settling behaviour, there were no significant differences between males and females in their attraction to yeast species, and there was suggestive evidence for genetically based differences in settling behaviour (BARKER et al. 1981). Laboratory experiments by ARMSTRONG (1992), using the same four isofemale lines and four pairs of yeast species as noted earlier, showed a generally positive association between long distance attraction (wind tunnel olfactometer) and OSP. Thus males and females with similar genotypes affecting OSP may be attracted to the same rot, where mating would occur. Even if some of these females had mated previously, *D. buzzatii* females are known to mate repeatedly, with most progeny following a remating being sired by that male (BUNDGAARD and BARKER 1996, unpubl.).

Fourthly, the models assume a single locus affecting habitat selection, with this locus or one or two other loci affecting fitness. The genotype-specific OSP found here, however, is apparently polygenic, given the differences among line combinations for the four lines with contrasting Pc vs. Crc preferences in additive, dominance and epistatic effects (Table 20). The genetic effects estimated from the generation means analyses (experiment 7) were very different for OSP and for fecundity (compare Tables 20 and 21), so that different polygenic loci in fact do determine OSP and fitness. While all models assume habitat selection is under simple genetic control (single locus), HEDRICK (1990a) notes that his model is robust for small selective differences, and that subtle genotype-specific habitat selection, even for polygenic traits, may be of major importance in the maintenance of genetic polymorphism. For *D. buzzatii*, polygenic control of OSP and fitness, with linkage disequilibrium between these two sets of loci, may be expected to maintain variation not only at these loci, but also at other loci linked to them.

In experiments 6–8, which were designed to determine the magnitude of genetic variation for OSP, and its possible genetic basis, only two yeast species (Pc and Crc) were used. These two species were chosen because (i) they are among the five most abundant yeasts isolated from cactus rots in Australia (BARKER et al. 1984), (ii) *D. buzzatii* females generally show a high preference for ovipositing on Pc and a low preference for Crc (VACEK et al. 1985; BARKER 1992), and (iii) significant isofemale line heritabilities had been demonstrated for the proportion of eggs laid on Pc when females were offered a choice of these two species (BARKER 1992; BARKER et al. 1994).

However, genetic variation in OSP may not be present for all pairs of yeast species, and that observed for a particular pair may differ when other yeasts are present. When females were given a choice among five yeast species (BARKER 1992), heritabilities of individual female preferences for each yeast (i.e.

proportion of eggs laid on yeast i vs. the other four) were significant for Pc, Crc and *Candida mucilagina*, but not for Cs and Clo. When females were given a choice between two yeast species (BARKER et al. 1994), significant genetic variation was found for Pc/Crc and for Crc/Clo, but not for Pc/Clo. However, when females were given a choice among all three species, the heritability of individual preferences for each yeast was significant. Further, BARKER et al. (1994) concluded that interactions among the yeasts apparently affect oviposition preferences, and that the proportions of eggs laid on each yeast are not directly predictable from the pairwise preferences. This aspect of interaction among yeast species affecting preferences was extended here in experiments 4 and 5 by further comparisons of two-way and three-way choice, and of mono- vs. bicultures.

For the 10 two-way choice combinations, differences among combinations were significant (Table 7), with the mean proportion of eggs laid on the first named yeast ranging from 0.352 for Cs/Pc to 0.804 for Cs/Crc. However, the four lines within each of the nine populations ranked differently for different combinations, with this primarily due to combinations including Cs (significant lines (populations) × yeast combinations (Table 8)). That is, there are significant genotype (line) × environment (yeast combinations) interactions, which would contribute, over and above habitat selection, to the maintenance of genetic variation (GILLESPIE and TURELLI 1989). Further significant G × E interactions are apparent in the comparisons of two-way and three-way choice (Table 10), where for some yeast combinations, the ranking of lines for preference of yeast i vs. yeast j varied depending on which third yeast was present.

The overall preferences for the five yeast species were the same in the two-way and three-way choice experiments, viz. Pc > Cs ≅ Pb > Clo > Crc. However, not all two-way or three-way combinations follow this ranking, adding further complexity to the interactions inherent in the system. In the two-way tests, Clo is exceptional (Table 8) with Clo > Cs and Crc > Clo, while in the three-way tests (Fig. 2), Clo and to a lesser extent Cs do not always fit the overall rankings.

In previous experiments (BARKER 1992), no genetic variation was detected for preferences for Cs or Clo. Although significant genetic variation was found for Clo/Crc by BARKER et al. (1994), they suggested that the apparent absence of genetic variation for Cs and Clo may be due to their fermentation ability and high levels of ethanol produced, and that the ethanol may well be a major component contributing to long-distance attraction to rots. However, in the more comprehensive two-way and three-way choice experiments here, each of the five yeast species is included in one or more combinations that exhibit significant genetic variation.

Additional tests of potential interactions among yeast species affecting female preferences were done in the comparisons of mono- and bicultures. In general, where a particular yeast is preferred in a two-way monoculture choice, it is also preferred over the biculture of that yeast and any other. In natural rots therefore, females could discriminate varying concentrations of yeast species, and oviposit in areas with

Table 22. *Means (back-transformed) of the high Pc preference lines (IG2 and T9) and low preference lines (MB6 and T3) for the fitness components measured in Experiment 9 (means with the same letter superscript within columns are not significantly different—Scheffe's test)*

Line	Developmental time (days)			Line	Viability (back-transformed)
	Males	Females	Overall		
MB6	16.3[a]	16.5[a]	16.4[a]	IG2	0.743[a]
IG2	15.7[b]	15.6[b]	15.7[b]	MB6	0.711[a,b]
T9	15.3[c]	15.3[b,c]	15.3[b]	T3	0.703[b]
T3	14.6[d]	14.9[c]	14.8[c]	T9	0.642[c]

Line	Female average body wt (back-transformed—mg)	Line	Average egg no. (back-transformed)	
			Per day of female life	Per test day
MB6	0.274[a]	IG2	2.69[a]	2.08[a]
IG2	0.259[a,b]	T9	1.53[b]	1.22[a,b]
T3	0.253[a,b]	T3	1.25[b]	1.18[a,b]
T9	0.237[b]	MB6	0.77[b]	0.65[b]

the highest concentration of their preferred yeast species.

In their review of the choice of hosts by ovipositing females, COURTNEY and KIBOTA (1990) conclude "that genetic variance for host use is widespread, even the rule" and adding "polygenic variation does seem to be present when it is looked for." Our genetic analyses (experiments 6–8) demonstrate genetic variance for OSP that is certainly polygenic. More importantly, however, the heritabilities (Table 13) and the generation means analysis (Table 20) show that much of the genetic variance is non-additive (dominance and epistatic interactions). This result, which relates specifically to the Pc/Crc choice, indicates that natural selection for increased Pc preference has largely depleted the additive genetic variance. The results of the selection experiment (experiment 8, Fig. 4) completely accord with this interpretation, as the realized heritability of index values for increased Pc preference was not significantly different from zero, whereas that for decreased Pc preference was 0.425 ± 0.093 ($P < 0.001$). We do not have similar comprehensive data for other pairwise tests of yeast species. However, given the results of BARKER (1992, simultaneous choice of five yeast species), and of the two-way and three-way choice and mono- vs. bicultures here, we would expect similar findings.

CONCLUSION

Previous studies of genetic variation in OSP have considered spatially separate resources, such as host plant species, alternative fermenting substrates or novel hosts (reviewed by JAENIKE and HOLT 1991). Here we have dealt with a more complex situation, where the alternative oviposition sites are patches of essentially adjacent or even partially mixed growing yeast species within the confines of a cactus rot. This yeast community may comprise up to five yeast species, and the species present vary from rot to rot. The OSP exhibited by individual females is not absolute, but may vary depending on which combination of yeast species is present in a rot. Nevertheless, genetic variation appears to be ubiquitous, polygenic and largely non-additive for all yeast combinations. Comparisons of our results with the assumptions of various models of habitat selection indicate that all assumed mechanisms that would contribute to the maintenance of genetic variation are met.

Thus OSP for yeast species would seem to be a powerful force for the maintenance of genetic variation, and not only at loci affecting the choice of oviposition sites.

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