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Allozyme Genotypes of *Drosophila buzzatii*: Feeding and Oviposition Preferences for Microbial Species, and Habitat Selection

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Abstract

Mature, mated female *D. buzzatii* were given a choice of nine microbial communities actively growing on cactus homogenate in laboratory population cages, and tests were made to determine if flies of different genotypes (for seven allozyme loci) chose different microorganism species for either feeding or oviposition. Variation in feeding preferences was determined from assays of electrophoretic genotypes and the ingested microorganism species of individual flies. Oviposition preference variation was analysed indirectly by assaying the genotypes of individuals raised from eggs laid on different microorganisms. No significant evidence was found for differences in feeding preferences among adults of different genotypes. For oviposition preferences, there were significant microorganism–genotype associations for each of seven polymorphic loci. Analyses of the total electrophoretic genotype, rather than of individual loci, showed that the genotypes of eggs laid on the same microorganism species were more similar than those laid on different species. That is, females of different genotypes show habitat selection for oviposition sites, which would facilitate the maintenance of genetic polymorphisms.

Introduction

Drosophila larvae and adults feed on microorganisms, and it is generally accepted that yeasts are an important component of the diet (Begon 1982). However, not all yeast species provide adequate nutrition (Wagner 1944, 1949; Vacek 1982), and a number of *Drosophila* species are able to discriminate among yeast species, and to exert specific preferences (Vacek *et al.* 1985, and references therein).

The cactophilic *Drosophila*, including *D. buzzatii*, provide a valuable model system for analysis of fly–microorganism interactions, as their natural feeding and breeding sites are known (rotting arms, cladodes and fruit of one or more cactus species), and can be assayed for microorganism content. Further, both the *Drosophila* and the microorganisms can be studied in the laboratory, and potentially, could be manipulated in natural populations. Yeasts are an important microbial component in cactus rots (Starmer *et al.* 1982; Barker *et al.* 1984), although little is known of other components of the microbiota such as bacteria and moulds.

Our studies of *D. buzzatii* have attempted to determine and understand mechanisms maintaining genetic variation at enzyme loci in natural populations. Some genetic variables (allele and genotype frequencies at six polymorphic loci) have shown significant associations with microorganism variability (yeast species) and microorganism mediated variability (e.g. alcohols) in the rots (Barker 1982). These and other associations with rot variables indicate that natural selection is affecting allozyme frequencies and, further, suggest environmental heterogeneity and diversifying selection as one important mechanism for the maintenance of genetic variability.

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Theoretical analyses show that environmental heterogeneity in space or in time can maintain genetic polymorphisms (Hedrick *et al.* 1976; Karlin 1982), although Maynard Smith and Hoekstra (1980) and Hoekstra *et al.* (1985) have shown that for some models of natural selection in heterogeneous environments, the conditions for the maintenance of polymorphism may be stringent. However, if individuals of different genotypes differ in habitat selection and tend to choose those parts of the environment where they are most fit, the conditions for maintenance of polymorphisms are relaxed (Taylor 1976).

Access to food of sufficient quantity and quality is a major aspect of the environment for any organism so that heterogeneity in food resources would seem *a priori* a likely candidate as a basis for habitat selection. For *D. buzzatii*, available evidence suggests that there is potential for such habitat selection. Firstly, *D. buzzatii* has been shown to discriminate among cactophilic yeasts in field experiments (Barker *et al.* 1981*b*) and in the laboratory (Vacek *et al.* 1985). Secondly, *D. buzzatii* females prefer for oviposition those yeast species that in general are best for larval development (Vacek 1982; Vacek *et al.* 1985). Thirdly, cactus rots are spatially and temporally heterogeneous in both abundance and frequency of different cactophilic yeasts (Barker *et al.* 1983; Barker, Starmer and Vacek unpublished data). Thus, *D. buzzatii* females in natural populations may discriminate among these rots for feeding and/or oviposition. Fourthly, an analysis of population structure in time and microgeographic space in one natural population (Barker *et al.* 1986) suggests that microspatial heterogeneity among individual rots affects genetic variability.

Nevertheless, the question remains—can genes choose habitats? (Jones 1980) or, more specifically, do adults of different genotypes for enzyme loci in *D. buzzatii* choose different microorganism species for either feeding or oviposition? In this paper, we report the results of an experiment designed to answer this question. Variation in feeding preferences was determined from assays of electrophoretic genotypes and ingested microorganism species of individual flies. The genotypes of ovipositing females could not be measured directly, so oviposition preferences were analysed indirectly by assaying the genotypes of individuals raised from eggs laid on different microorganisms. These data were obtained as part of the experiment of Vacek *et al.* (1985), which considered feeding and oviposition preferences at the species level.

Materials and Methods

The experiment was designed to give *D. buzzatii* females a choice of nine different microbial communities for feeding and oviposition over a 3-day period. The nine communities were bacteria alone (at least four unidentified species isolated from the rots from which *D. buzzatii* had been collected for these experiments) and bacteria plus one of eight yeast species (Table 1) growing on 3–4 mm thick slabs of 10% cactus (*Opuntia stricta* cladode) homogenate, 1·5% agar medium. Full details of the flies and microorganisms used and experimental design and procedures are given by Vacek *et al.* (1985).

Flies that emerged from 16 rots collected at Hemmant, Queensland (locality 31 of Barker and Mulley 1976) were set up (177 vials, 5 pairs of flies per vial) on autoclaved agar–sucrose–yeast (*Saccharomyces cerevisiae*) medium (Barker *et al.* 1981*a*). Progeny of these matings were collected over a 5-day period and aged on the same medium (10 pairs per vial), with transfer to fresh vials every 2 or 3 days during this aging period. On the day before initiation of the experiment, 90 of these mated females (average 7 days old) were placed into each of 27 plastic cages (22 by 22 by 7·5 cm with nine holes in a 3 by 3 matrix in the base for medium jars) containing agar–sucrose–yeast medium. Cages were set at random on shelves of one wall of a walk-in incubator maintained at 25 ± 0·5°C and a 12 h light, 12 h dark cycle.

On day 1 (experimental initiation) at 0730 h, discs 2·7 cm in diameter were cut from each of the microbial cultures (previously incubated for 48 h), and placed into 2·8 cm diameter, 3–4 mm deep depressions cut in the smaller ends of corks which were inserted into the nine holes in the base of each cage. To minimize cross-contamination among discs by fly movement, discs were replaced with new 48 h growth at 1200, 1830, and 2230 h on day 1. On days 2 and 3, discs were renewed at 1000 and 2000 h only, because the 0730 and 1830 h renewals were likely to interfere with peak oviposition. The increased intervals were not long enough to allow contaminants to competitively multiply on the heavy initial growth.

The nine microbial communities were assigned to fixed positions in each cage according to a 9 × 9 Latin square design, i.e. one Latin square for each of three sets of nine cages. Thus, in a set of nine cages each community was present once in each cage, but in a unique position in each, ensuring that treatments were interspersed over space among cages.

Table 1. Taxonomic designations of yeast species, code, Davis stock numbers, and abundance in rots of *Opuntia stricta*

Yeast species	Code[A]	Davis No.[B]	Abundance[C] (%)
Candida sonorensis (Miller *et al.*) Meyer & Yarrow comb. nov. 1978	Cs	77–38	24·4
Pichia cactophila Starmer *et al.* 1978	Pc	77–168	21·9
Clavispora sp. O[D]	ClO	77–182	16·8
Pichia sp. B[E]	PB	77–207	4·8
Cryptococcus cereanus Phaff *et al.* 1974 complex	Crc	77–141	3·9
Candida mucilagina Phaff *et al.* 1980	Cm	76–234C	3·2
Rhodotorula minuta (Saito) Harrison var. *minuta* 1922	Rhm	78–284	2·5
Pichia opuntiae Starmer *et al.* var. *opuntiae* 1979	Po	77–201	2·3
Bacteria	Bact	Not assayed	Not assayed

[A] In all subsequent tables, the codes are used in place of the name.
[B] Stock numbers of strains, held in Professor H. J. Phaff's laboratory, Department of Food Science and Technology, University of California, Davis, California.
[C] Frequency of these species in 944 isolates of 42 species reported by Barker *et al.* (1984).
[D] *Lodderomyces opuntiae* in Barker *et al.* (1981*a*).
[E] *P. cactophila* variety in Barker *et al.* (1981*a*).

Determination of Feeding Preferences

On the evening of each of the three days (1830 h on day 1 and 2000 h on days 2 and 3), all flies from one set of nine cages were removed and placed in vials on ice to minimize digestion. A random sample of 30 flies from each cage was assayed for yeasts consumed, and for allozyme genotypes at six loci, *viz.* esterase-1 (*Est-1*: EC 3.1.1), esterase-2 (*Est-2*: EC 3.1.1), *β-N*-acetylhexosaminidase (*Hex*: EC 3.2.1.52), phosphoglucomutase (*Pgm*: EC 5.4.2.2), aldehyde oxidase (*Aldox*: EC 1.2.3.1) and alcohol dehydrogenase-1 (*Adh-1*: EC 1.1.1.1). On day 3, a seventh locus [aminopeptidase (cytosol) = leucine aminopeptidase] (*Lap*: EC 3.4.11.1) also was assayed.

The flies were surface-sterilized in 70% (v/v) ethanol (Vacek *et al.* 1979) before homogenization in 0·4 ml polyethylene microcentrifuge tubes containing 30 μl sterile grinding buffer (0·01 M Tris-citrate, pH 7). A small inoculating loop full of homogenate was placed into one drop of sterile water on one-half of a plate of AYMA (Difco yeast extract–malt extract agar, acidified with 1 M HCl to pH 3·8–4·0), streaked, and incubated at 25°C for 4–6 days. The remaining homogenate was centrifuged and the supernatant divided equally on to two 4 by 7 mm filter paper wicks for use on each of two independent horizontal starch gels. Enzymes were assayed using the methods of Ayala *et al.* (1972, 1974) and Barker and Mulley (1976), with modifications as given by Barker *et al.* (1986).

Yeasts isolated from each fly were unambiguously identified by colony type and cell morphology, and up to 150 colonies per fly were counted for frequency determination.

All discs included the bacterial community, but the relative frequency of bacteria consumed was not determined. However, flies that had fed on bacteria only and not on any of the yeast discs could be distinguished, since some of the bacterial species grew on AYMA. Such flies were given a score of 100% bacteria and zero for each yeast species. Thus in the results we distinguish preferences for the eight yeast species or for bacteria only, but to avoid writing this out in full each time, will refer simply to yeasts.

To decrease the sampling error involved in determining relative frequency from a low total colony count, only flies with greater than 16 yeast colonies or those with no yeasts and greater than 16 bacterial colonies were considered. In all, 8% of the flies were excluded from the analysis on these criteria. Others were excluded because of extensive mould contamination (3%), no microbial growth (0·3%) or colonies too dense to count (1%). The maximum number deleted from the sample of 30 flies per cage was nine.

Determination of Oviposition Preferences

On each evening of days 2 and 3, for the set of nine cages terminated at that time, the discs with eggs were retained and kept in plastic boxes at 25°C. The objective was to raise and electrophoretically assay 11 adults from each yeast in each cage. To obtain these adults, 40 larvae were to be collected (<2 h old) from each disc and placed in a vial containing 6 ml agar–sucrose–yeast medium. However, the numbers of eggs per disc on day 2 were about one third the numbers on day 3 (Vacek *et al.* 1985), and no or very few eggs were laid on the less preferred yeasts. On day 2, 20% of the discs had less than five eggs, and a further 47% had less than 40 but more than five. Corresponding values for day 3 were 14% and 21%. For those discs with more than five but less than 40 eggs, all larvae were collected. Thus on day 2 (day 3), 41% (21%) of the discs produced no adults for assay, while 15% (11%) produced less than 11. More than 11 adults were assayed for some other cage-yeast combinations, so that an average of 12·6 adults were assayed for 48 cage–yeast combinations on day 2, and 13·5 adults for 62 cage–yeast combinations on day 3.

All loci are autosomal, and *Est-1*, *Est-2*, *Aldox* and *Lap* have been located to chromosome II, but the linkage distances are not known. *Pgm* is on chromosome IV (A. Fontdevila, personal communication), *Adh-1* is on chromosome III (by homology with *D. mojavensis* Zouros, 1976), and *Hex* is on either chromosome IV or V.

Table 2. Allele frequencies for each of the seven loci, estimated from the 30 experimental flies assayed from each of the 27 cages

Locus	Allele				
	a	*x*	*b*	*c*	*d*
Pgm	0·017		0·981	0·002	
Aldox	0·958		0·042		
Est-1	0·233	0·016	0·644	0·107	
Est-2	0·444		0·240	0·113	0·203
Hex	0·887		0·113		
Adh-1			0·749	0·251	
Lap	0·093		0·850	0·057	

Allele frequencies (Table 2) for all loci except *Adh-1* were such as to give small expected numbers for some phenotypic classes. Thus for canonical correlation and log-linear analyses, phenotypic classes were pooled as follows:

Locus	Alleles	Classes
Aldox	*a*, *b*	*aa* (*ab* + *bb*)
Hex	*a*, *b*	*aa* (*ab* + *bb*)
Lap	*a*, *b*, *c*	*ab*, *bb* (pool of remaining classes)
Pgm	*a*, *b*, *c*	*bb* (*ab* + *bc*)
Est-1	*a*, *x*, *b*, *c*	*ab*, *xb*, *bb* (pool of remaining classes)
Est-2	*a*, *b*, *c*, *d*	*aa*, *ab*, *ac* (pool of remaining classes)

Statistical analyses were done using the BMDP (1983) and SPSS Inc. (1983) packages.

Results*

In the analyses of associations between fly genotype and the yeasts on which it had fed, yeast frequency was defined in two ways: (1) the actual frequency of each yeast isolated from each fly, or (2) the most common yeast in each fly was scored as having a frequency of unity if its actual frequency was greater than 50%. This latter procedure eliminated yeasts at low frequency in individual flies, and hence any spurious effects due to inclusion of yeasts ingested only during exploratory behaviour of the different feeding substrates.

Canonical correlation analyses were done separately for each day, to determine any overall patterns of association of genotypes at each locus with the yeasts. In these, the actual frequency of each yeast in each fly was used, and cage was included as a variable in the yeast set. Only the correlation for day 2 (0·443) was significant ($P < 0·01$), but the variance explained by the canonical variables was only 3·2% for the genotypes and 2·1% for the cage and yeasts.

Log-linear analyses, including the variables day, cage, yeast and genotype, done separately for each locus and using both ways of defining yeast frequency, showed no significant genotype–yeast associations, nor were any of the higher order associations including yeast and genotype significant. There is therefore no indication of yeast feeding preferences by adults of different genotypes.

Table 3. Nature of the yeast–genotype associations for each locus, as shown by the individually significant standardized deviates
$*P < 0·05$; $**P < 0·01$; $***P < 0·001$

Locus	Obs. > Exp.	Obs. < Exp.
Pgm	Cm (*ab* + *bc*)*	Rhm (*ab* + *bc*)*
Aldox	Cs (*ab* + *bb*)*	
Est-1	Crc (*xb*)**	PB (*xb*)**
	Bact (*ab*)*	Rhm (*ab*)*
	Cm (*ab*)**	Cm (*bb*)*
Est-2	PB (*ab*)***	PB (*ac*)**
	Cm (*aa*)*	Cs (pool)**
	Crc (*ab*)**	ClO (*ab*)*
Hex	ClO (*ab* + *bb*)**	Cs (*ab* + *bb*)*
	Bact (*ab* + *bb*)*	
Adh-1		Crc (*cc*)***
Lap	ClO (pool)*	
	Crc (*ab*)***	Crc (pool)*

No. of times each yeast was involved in a significant association[A]

Cs	Pc	ClO	PB	Cm	Po	Rhm	Bact	Crc
3	–	3	2	3	–	2	2	4

[A] Concurrent effects on two genotypes at the one locus were counted as one association (e.g. Cm and *Est-1*).

For oviposition preferences (determined by assaying flies emerging from eggs laid on each yeast), log-linear analyses showed significant yeast–genotype associations for each of the seven loci, and the significant standardized deviates (Table 3) show

*The original data have been deposited with the Editor-in-Chief, Editorial and Publications Service, 314 Albert St, East Melbourne, 3002, and copies are available on request.

that all but two of the yeasts (*P. cactophila* and *P. opuntiae*) contributed to these associations, and up to five yeasts were involved for any one locus (*Est-1* and *Est-2*). However, little weight can be attached to the specific significant deviates for each separate locus, except as guides for further experimental analysis. If the allozyme genotype of a female affects her preferences for oviposition on particular yeasts, as these results suggest, then the apparent preference for one yeast dictated by her genotype at one locus might be confounded by preference for some other yeast dictated by her genotype at another locus.

Thus while it is perhaps surprising that significant associations have been detected, it is apparent that the overall genotype, rather than the separate loci, also should be considered. Thus analyses were done to determine if there were significant associations between the overall genotype and yeasts. Again it should be emphasized that the question of interest is whether females of different genotypes show preferences for particular yeasts as oviposition sites. By using the genotypes of flies raised from eggs laid on the different yeasts, these analyses must be a less sensitive measure of any such association, because of segregation of different alleles of heterozygotes in the eggs of each female, and the contribution of the male parent to the embryo.

Table 4. Canonical correlations of genotypes at each of seven loci in flies emerging from eggs laid on each yeast with the variables yeast, day and sex

$^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$

Variable	Canonical correlations and coefficients		
	First	Second	Third
Pgm	0·09	0·43	0·07
Aldox	**0·87**	−0·30	−0·25
Est-1	0·13	0·07	**−0·59**
Est-2	−0·12	−0·001	0·11
Hex	0·34	**0·75**	0·23
Adh-1	−0·03	−0·18	−0·05
Lap	0·28	−0·29	**0·73**
Yeast	**−0·50**	**0·77**	−0·39
Day	−0·37	**−0·60**	**−0·71**
Sex	**0·76**	0·22	**−0·61**
R	0·19***	0·12***	0·12**

Two analyses were done to test for association between overall genotype and yeasts. Firstly, canonical correlations between genotypes at each of the seven loci and the variables yeast, day and sex (Table 4) gave three significant correlations, with high canonical coefficients for yeast in the first two. Although significant, these canonical correlation coefficients were small, and the variance explained by the canonical variables was only 2·2% for the genotypes and 1·0% for yeast, day and sex.

For the second analysis, genotype similarity indices for individuals from each cage were calculated (1) between individuals which were laid on the same yeast (within yeast) and (2) between individuals which were laid on different yeasts (between yeast). If there were an association between overall electrophoretic genotype and yeast species, the eggs laid on a particular yeast would be expected to be genetically more similar to each other than to eggs laid on any other yeast, i.e. higher within-yeast than between-yeast indices. The within-yeast index was obtained by comparing the

genotypes at each locus for each fly with every other fly from the same yeast, expressing the index as (number of same genotypes ÷ number of loci assayed for both flies), and calculating the mean similarity index and its variance for each yeast. The between yeasts index was obtained similarly, but comparing for all possible pairs of different yeasts, the genotypes of each fly from yeast (*i*) with every fly from yeast (*j*). Thus for each cage, up to nine within-yeast indices and up to 36 between-yeast indices could be estimated. However, because no or very few flies were raised and assayed from some yeasts, the average numbers of within-yeast and between-yeast indices per cage were 6 and 17, with ranges from 3 to 8 and 3 to 28 respectively. Therefore, the analyses of variance presented were done using cell means as the unit of observation (i.e. averages of the within- and between-yeast indices in each cage, and the average of their variances). These analyses minimize any possible bias due to the different numbers of observations for the within-yeast and between-yeast indices, and any effects of heterogeneity of within-cell variances.

Table 5. Analyses of variance of the mean similarity indices within and between yeasts for each cage, and of their average variances
$*P < 0{\cdot}05$; $***P < 0{\cdot}001$

Source of variation	Degrees of freedom	Mean square	
		Index	Variance of index
Cages (C)	17	0·00123***	0·0000094*
Within *v.* between (WB)	1	0·00612***	0·0000212*
C × WB (error)	17	0·00014	0·0000040

Although there were significant differences among cages in the mean similarity indices (Table 5), the mean within-yeast index was higher than the mean between-yeast index in all cages but one, and over cages, the difference was highly significant. The ranges for these within-cage indices were 0·580–0·658 for within yeast and 0·538–0·630 for between yeast, with overall means of 0·615 and 0·589 respectively. Variances of the within-cages indices also were significantly different among cages (Table 5), and higher for within yeast, as compared with between yeast (overall means of the variances being 0·0267 and 0·0251 respectively). Although these variances were heterogeneous, there was no association between variances and means, either for within yeasts or between yeasts. Analyses using all observations also were done, and gave the same results, with the exception of a higher level of significance ($P < 0{\cdot}001$) for the cages effect on the variance of the index.

In a one-way analysis of variance (Table 6) of the within-yeast indices (treating cages as replicates), the variation among yeasts was almost significant ($P = 0{\cdot}088$), with average values ranging from 0·580 for ClO to 0·676 for Cm.

However, only small numbers of eggs were laid on some discs, particularly on day 2. As 7–10-day-old *D. buzzatii* females lay an average of 40–50 eggs per day (Barker and Fredline 1985), the eggs laid on any one of the less preferred yeasts could have been produced by one female. If this were so, one would expect higher similarity within yeasts than between yeasts. Further analyses were done to evaluate this possibility.

Firstly, if within-yeast similarity were primarily a function of the numbers of females laying on each yeast, a negative correlation would be expected between the number of eggs laid and the within-yeast similarity. The correlation coefficients for

day 2, day 3 and overall were $-0{\cdot}124$ (d.f. = 44, $P = 0{\cdot}412$), $0{\cdot}004$ (d.f. = 60, $P = 0{\cdot}974$) and $-0{\cdot}103$ (d.f. = 106, $P = 0{\cdot}288$).

Secondly, the similarity indices already presented were calculated within cages. If the eggs laid on a particular yeast are genotypically more similar than those laid on different yeasts, the indices should still differ when estimated between cages.

Table 6. Mean within-yeast similarities for each yeast calculated within cages and between cages, and analyses of variance
***$P < 0{\cdot}001$

Within cages			Between cages (within days)		
Yeast	Mean (s.d.)	n^A	Yeast	Mean (s.d.)	n^A
Cm	0·676 (0·078)	5	Cm	0·660 (0·047)	4
Crc	0·663 (0·066)	7	Bact	0·621 (0·040)	29
Rhm	0·630 (0·084)	12	Crc	0·618 (0·052)	11
Bact	0·627 (0·060)	10	Rhm	0·596 (0·050)	30
Cs	0·610 (0·060)	17	Cs	0·596 (0·041)	64
PB	0·599 (0·069)	12	PB	0·580 (0·041)	42
Pc	0·597 (0·059)	18	ClO	0·577 (0·045)	46
Po	0·593 (0·038)	14	Pc	0·572 (0·050)	72
ClO	0·580 (0·105)	14	Po	0·570 (0·027)	46

Source of variation	Within cages		Between cages	
	d.f.	Mean square	d.f.	Mean square
Between yeasts	8	0·0088	8	0·0137***
Within yeasts	100	0·0049	335	0·0019

[A] Number of observations.

A within-yeast (between-cages) similarity index was calculated as previously, but comparing the genotypes for each fly from yeast (i) in cage (k) with every fly from yeast (i) in cage (l), then calculating the mean index and its variance for each yeast in each pair of cages. The between-yeast (between-cages) index was obtained similarly,

Table 7. Analyses of variance of the mean similarity indices within and between yeasts (both between cages), and of their average variances
*$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$

Source of variation	Degrees of freedom	Mean square	
		Index	Variance of index
Days (D)	1	0·00875***	0·0000589***
Within *v.* between (WB)	1	0·00061*	0·0000002
D × WB	1	0·00010	0·0000018
Cage pairs (C) within days	70	0·00087***	0·0000084**
Error	70	0·00015	0·0000047

but comparing for all possible pairs of different yeasts, and for each pair of cages, the genotypes of each fly from yeast (i) in cage (k) with every fly from yeast (j) in cage (l). Because of the smaller numbers of eggs laid on each yeast on day 2, these between-cages indices were calculated separately for the nine cages of day 2 and the nine of day 3.

Analyses of variance were done using the cell means as the unit of observation, for the same reasons as for analysis of the within-cage indices. There were significant differences among cage pairs within days for both mean and variance of the indices (Table 7), but the mean within-yeast (between cages) index was significantly higher than the mean between-yeast (between cages) index. The former was higher than the latter for 23 of 36 cage pairs on day 2 and for 25 of 36 on day 3. As with the within cage indices (Tables 5), there was no association between variances and means, and analyses using all observations gave the same results, except for a higher level of significance ($P < 0{\cdot}01$) for the effect of within *v.* between indices, and a significant effect ($P < 0{\cdot}05$) for days × within versus between variances.

One-way analysis of variance of the within-yeast (between-cages) indices (Table 6) showed highly significant differences between yeasts, with means for each yeast that were less than the within-cages indices, but which showed a similar ranking to the latter.

The pattern of results for the within-cages and between-cages indices are the same. In both cases, the mean within-yeasts index was significantly greater than the mean between-yeasts index, and the rank orders of the mean within-yeasts indices were similar.

Discussion

The results show that adults of different genotypes for the seven polymorphic enzyme loci do not have any preferences for feeding on particular yeasts. However, females of different genotypes apparently prefer particular yeasts for oviposition.

As oviposition preferences were determined by assaying the genotypes of adults raised from eggs laid on each yeast, factors other than direct female choice conceivably could contribute to the apparent preferences, e.g. variation among genotypes in fecundity or in egg to adult survival. If females of different genotypes differed in fecundity, but all females oviposited on the yeasts at random, mean genotype frequencies should be different between the adult population and the eggs laid, but there would be no concomitant variation in genotype frequencies of eggs laid on different yeasts. Similarly, differences among genotypes in egg to adult survival should lead to different genotype frequencies in the eggs laid, as compared with the adult population, but could give different genotype frequencies on different yeasts only if there were differences among genotypes in survival that varied depending on the yeast on which the eggs were laid. This does not seem probable as all eggs were treated in the same way, larvae were collected immediately after hatching, and all allowed to develop to adults under standard, uncrowded conditions.

In order to evaluate possible variation among genotypes in either female fecundity or egg–adult survival, G-statistic tests were done comparing the genotype frequencies at each locus in each cage between the adult population and the progeny (flies raised from eggs). For *Lap*, only seven cages on day 3 had been assayed for the adult population, and none of these tests was significant. *Pgm*, *Hex* and *Est-2* each had one significant test in the 18 cages, *Aldox* had two, *Adh-1* four, and *Est-1* five. The differences in genotype frequency between the two generations were not consistent over the four significant cases for *Adh-1*, and the only evidence for directional frequency changes was for *Est-1*, where in all five significant cases, the frequency of *ab* was less than expected in the progeny generation.

Thus we conclude that the apparent oviposition preferences detected by the various single-locus and multi-locus analyses do indeed reflect choice by females of different genotypes for oviposition on the different yeasts.

Two of the yeasts, *P. cactophila* and *P. opuntiae*, which were not involved in significant yeast–genotype associations and which had low values for the within-yeast similarity indices, were the first and third most attractive for oviposition (Vacek *et al.* 1985). The strong oviposition preference for *P. cactophila*, most likely due to its being the only one of the species included here producing ethyl acetate (Fogleman 1982), which is a general attractant, may have precluded the expression of any differential preferences by different genotypes. The same argument cannot apply to *P. opuntiae*, however, as Fogleman (1982) found this species produced less volatiles than any of the other cactophilic yeasts that were studied. *P. opuntiae*, however, may represent the opposite extreme (i.e. no volatile cues), which would evoke no discrimination by genotypes.

On the other hand, those yeasts which contributed most to the yeast–genotype associations and which had high values for the within-yeast similarity indices, were generally those that had fewest eggs laid on them (i.e. were less attractive for oviposition), particularly *Cr. cereanus*, *C. mucilagina*, *Rh. minuta* and bacteria. As the genetic similarity indices estimated between cages were significantly higher for within yeasts than between yeasts (Table 7), the associations detected cannot be a function of the smaller numbers of eggs laid on these yeasts. Although less attractive to *D. buzzatii* in general, these yeast species are preferred for oviposition by some genotypes.

Consideration of the known physiological profiles (Starmer *et al.* 1982) of the yeasts included in this study provides little indication of the possible basis for the oviposition preferences of the different allozyme genotypes. One reason for this is the lack of information on the natural substrates of the *Drosophila* enzymes. Nevertheless, the significant association of *Adh-1* genotypes with *Cr. cereanus* may result from the ability of this species to utilize 2-propanol. However, it is not necessary to assume that these results indicate the allozyme genotypes themselves to be the basis of habitat selection. If some other locus (or loci) showed genetic variation for habitat selection with respect to yeast species (e.g. genetic variation in olfactory responses—Hoffmann *et al.* 1984), and if genotypes at an allozyme locus differed in fitness on the different yeast species, then the 'habitat selection' genotype for a particular yeast would become associated with the allozyme genotype having highest fitness on that yeast. Thus an association of allozyme genotype with habitat would be expected. As the flies used in the experiment were all raised in the laboratory on a killed yeast (*Saccharomyces cerevisiae*) medium, and had not been exposed to the cactophilic yeasts before introduction to the cages, we have demonstrated habitat selection based on the genotype. In natural populations, this could be augmented by habitat selection due to conditioning, i.e. females tending to return for oviposition to the yeast species on which they developed as larvae.

Although the mechanisms cannot yet be defined, the demonstrated preferences of different genotypes for oviposition on different yeasts strengthen the possibility of genetic heterogeneity due to habitat selection, and they emphasize the need, and provide hypotheses, for detailed experimental study. Habitat selection would be implicated if eggs of the different genotypes were laid in rots containing the yeast species which best suited their growth and survival as larvae. In addition, as most

cactus rots have two or more yeast species present (Barker *et al.* 1984), further partitioning of the yeast species food resource by larvae would accentuate the habitat selection.

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