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Attraction of *Drosophila buzzatii* and *D. aldrichi* to Species of Yeasts Isolated from their Natural Environment. II.* Field Experiments

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Abstract

In three experiments done in one natural population over an 18-month period, *D. buzzatii* showed differential attraction to five yeast strains isolated from rotting *Opuntia inermis*, but the relative attractiveness of the yeasts was not the same in all experiments. There were indications that these changes in attractiveness could be related to the relative frequencies of the yeasts in natural rots at the time of each experiment. The differential yeast preferences of *D. buzzatii* and *D. aldrichi* found in one experiment indicate a degree of niche separation, which may be dependent on separate *Drosophila*–yeast evolution in the regions of origin on these *Drosophila* species, or may still be evolving in Australian populations. Different genotypes, at least at the *Esterase-2* locus, are differentially attracted to the yeast strains, so that nutritional variation may contribute to within-population selection and the maintenance of allozyme polymorphism.

Introduction

Several studies of *Drosophila* species in their natural environment have shown that they are differentially attracted to different species of yeasts, and that there are differences among *Drosophila* species in the yeast species to which they are attracted (da Cunha *et al.* 1951; Dobzhansky and da Cunha 1955; Dobzhansky *et al.* 1956; da Cunha *et al.* 1957; Kaneko 1960). It is likely therefore that all *Drosophila* species that breed and/or feed in rotting vegetable matter have the ability to discriminate among yeast species.

Drosophila buzzatii and *D. aldrichi* are cactophilic species of the *mulleri* subgroup that breed and feed in rotting cladodes of a number of species of *Opuntia*. As both species have been commonly found breeding in the same *Opuntia* rot in Australia (Mulley and Barker 1977), differential choice of yeast species is likely to contribute to niche separation and reduction in interspecific competition. However, as these species are known to be sympatric only in Australia, and have been so only for some 50–60 years (Barker and Mulley 1976), such niche separation may not be well-developed and may still be evolving. While this raises interesting possibilities for experimental analysis, our studies of these two species have been concerned primarily with the mechanisms maintaining genetic variation at allozyme loci in natural populations (Barker 1977; Mulley *et al.* 1979). The particular advantage of these species is that they are apparently specific to the cactus niche, so that their breeding and feeding sites are known and amenable to investigation.

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Although there are differences among *Drosophila* species in the yeasts to which adults are attracted, and also in the suitability of particular yeast species for the support of larval growth and development (Wagner 1944, 1949; Dudgeon 1954; Cooper 1960), less attention has been paid to the possibility of such differences among genotypes within a species. However, da Cunha (1951) and Dobzhansky and Spassky (1954) have shown that the adaptive values of inversion karyotypes in *D. pseudoobscura* varied when different yeasts and bacteria were used as food sources. Because of the importance of yeasts in the nutrition of *Drosophila*, assays of yeast species from rotting *Opuntia* were done. These showed a diversity of species among rots and at different times within a locality, and among localities (Barker 1977), so that differential preferences for and differential utilization of yeast species by different genotypes could contribute to within-population selection.

Laboratory experiments (Barker *et al.* 1981) have shown differential attractiveness of yeast species for young adults of *D. buzzatii* and *D. aldrichi* and have suggested niche separation between these species in the utilization of yeasts by mature females. The experiments reported here were designed to determine:

(1) Whether such differential attractiveness could be demonstrated in natural populations, and if so, whether the preferences of the flies were constant over time and different for the two species,

(2) Whether any demonstrated preferences were related to the relative frequencies of the tested yeast species in the locality at the time of the experiment, and

(3) Whether genotypes at any of the polymorphic allozyme loci were differentially attracted to different yeast species.

Materials and Methods

Three experiments were done with the five wild yeast strains used by Barker *et al.* (1981) for laboratory attractiveness studies. These strains were isolated from rotting cladodes of *Opuntia inermis* at 'Yarrawonga', Hunter Valley, N.S.W. (locality 5 of Barker and Mulley 1976), where these experiments also were done. The results of experiment 1 were discussed briefly by Barker (1977), where preliminary identifications of the yeast species were given. However, one strain identified there as *Pichia sp.* (Code No. Y6) was not used in these experiments. Final identifications of the other four strains and the correct fifth strain made by Professor H. J. Phaff and Ms M. Miranda were as follows (code numbers used are the same as in Barker *et al.* 1981):

Code No.	Code No. (Barker 1977)	Species
Y1(a)	Y4	*Candida sonorensis* Miller *et al.*
Y1(b)	Y5	*Candida sonorensis* Miller *et al.*
Y2	Y3	*Pichia cactophila* Starmer *et al.*
Y4	Y2	*Pichia cactophila* variety
Yx	—	*Cryptococcus albidus* (Saito) Skinner var. *albidus*

Y1(a) and Y1(b) initially were considered by us as different on the basis of colony and cell morphology, and were identified as two different *Candida* species by Centraalbureau voor Schimmelcultures (Barker 1977). As Y1(a) and Y1(b) were differentially attractive to *D. buzzatii* in experiment 2 and to *D. aldrichi* in experiment 3 (see Results, Table 3) and as the identifications done by Professor Phaff and Ms Miranda were done after experiment 3, it seems most likely that a mistake was made in strain maintenance after experiment 3 was set up. That is, for these experiments, Y1(a) and Y1(b) were two different *Candida* species, one of which was *C. sonorensis*. Photographs of cells taken at the time of experiment 1 show that the two strains had distinctly different cell morphologies, and that Y1(b) could be *C. sonorensis*, while Y1(a) most likely was not. *P. cactophila* variety may be a

new species (Phaff and Miranda, personal communication). It is similar to *P. cactophila* on a number of criteria, but distinguishable in some; in particular, *P. cactophila* variety is fermentative.

A sufficient amount of each strain was grown prior to each experiment as described by Barker *et al.* (1981) for their experiments 1 and 2. Once prepared, 8 ml suspension of each yeast (1 yeast paste: 2 distilled water) was added to 200 ml autoclaved mashed *O. inermis* cladodes in each of four sterilized 2 litre plastic containers (17 cm diameter, 9·5 cm deep; with clip on plastic lids having a 1 cm hole stoppered with cotton), and left at 25°C for 48 h (expt 1) or 24 hours (expt 2). For experiment 3, the final weight of each yeast produced was measured and all yeasts diluted to the same concentration of 0·68 g/ml with sterile distilled water. For each bait, 4·5 ml of this suspension was added to 200 ml autoclaved *O. inermis* (as above), and the baits were left at 25°C for 24 h. Laboratory studies have shown that these yeasts grow well on the autoclaved cactus.

The baits were transported to 'Yarrawonga' and set out for the experiment 72 h (or 48 h) after initial culture. In each experiment, our normal bait for collection (banana mash fermenting with *Saccharomyces cerevisiae*—coded YSc) also was used for comparison, but an autoclaved cactus bait as control was not included, as our interest was in the differential attractiveness of the yeasts.

Collections of *Drosophila* species have been done monthly at 'Yarrawonga' over four years, each collection being done at 10 defined sites. Four of these sites (Nos. 7–10), on average about 100 m apart, were used in each experiment. At each site, the six containers were placed in a random sequence at equal intervals on the circumference of a circle of approximately 3–4 m diameter. Six collections were made over a 3-h period in the late afternoon, the period of peak fly activity. These collections were made at 30-min intervals, and after each one, the containers at each site were put in a new random sequence. In experiments 2 and 3, the containers were covered with their lids and removed after the last collection, and then put out again the next afternoon, when the above collection procedure was repeated. Movement of flies between baits during the first collection period could cause contamination of the baits. However, given the film of growth of each test yeast over the surface of each bait, growth by any contaminant yeast in the intervening 24 h is likely to be insignificant.

The numbers of each sex of *D. buzzatii* and *D. aldrichi* at each collection for each yeast were recorded (except for expt 1 where collections were not kept separate), and the *D. buzzatii* collected in each experiment were assayed to determine gene and genotype frequencies for each of the six loci shown to be polymorphic by Barker and Mulley (1976), *viz. Esterase-1*, *Esterase-2*, *β*-N-*Acetylhexosaminidase* [previously called *Pyranosidase* (Barker 1980)], *Phosphoglucomutase*, *Aldehyde oxidase* and *Alcohol dehydrogenase-1* (except for *Alcohol dehydrogenase-1* males in expt 1 and *Aldehyde oxidase* in expt 2 where the assays failed). For each experiment, the numbers of each genotype and of each allele were classified by days (expts 2 and 3 only), sites, sex and yeast at which the flies were collected, and analysed by partition of χ^2. In experiment 2, some of the flies collected were not assayed for *Esterase-1*, *Esterase-2* and *Alcohol dehydrogenase-1*, so that analyses were possible for day 2 only for *Esterase-2*, and for sites 7 and 10 for *Esterase-1* and *Alcohol dehydrogenase-1*.

In addition, rotting *Opuntia* cladodes were sampled to determine the frequencies of wild yeasts present at the time of experiments 2 and 3. Approximately 1 g of rot tissue per cladode (one rot per plant) was diluted, plated and incubated at 25°C. Representatives of each morphological type in each sample were brought into pure culture by two or more successive platings. All cultures were maintained and later identified to species by Professor Phaff and Ms Miranda. Where two or more different cultures from the one sample were identified as the same species, they were considered a single isolate. In October–November, 1976 (expt 2), 72 isolates were obtained from 40 cladode rots, and in March–April, 1977, 92 isolates from 63 cladode rots.

Results

Yeast Attractiveness

The numbers of *D. buzzatii* and *D. aldrichi* collected and the dates of each experiment are given in Table 1. Other species collected were *D. hydei*, *D. immigrans*, *D. melanogaster*, *D. simulans*, *D. dispar*, *D. lativittata*, *D. enigma* and *D. subnitida*, but overall about 30% of flies of these species were collected from the banana bait. Sufficient *D. aldrichi* for meaningful analysis were collected only in April 1977, so comparison of the responses of the two species is restricted to this one experiment.

As the experimental designs were identical for experiments 2 and 3, valid statistical comparisons can be made of the responses of *D. buzzatii* in different seasons, while qualitative comparisons are made for experiments 1 and 2.

Table 1. Numbers of *D. buzzatii* and *D. aldrichi* collected at each site on each day in each of the three experiments

Day	Site	*D. buzzatii* ♂	*D. buzzatii* ♀	*D. aldrichi* ♂	*D. aldrichi* ♀
		Experiment 1—November 1975			
—	7	40	43	0	1
	8	77	63	2	2
	9	47	41	0	1
	10	62	55	4	0
Total		226	202	6	4
		Experiment 2—November 1976			
1	7	59	70	1	2
	8	123	103	6	2
	9	115	106	1	4
	10	130	104	5	7
Total		427	383	13	15
2	7	135	154	0	5
	8	184	142	1	3
	9	78	106	0	1
	10	112	80	2	0
Total		509	482	3	9
		Experiment 3—April 1977			
1	7	90	99	31	27
	8	62	70	26	25
	9	105	96	35	39
	10	48	47	7	18
Total		305	312	99	109
2	7	80	90	13	31
	8	72	82	21	18
	9	123	132	46	22
	10	38	51	7	9
Total		313	355	87	80

Considering first the attractiveness of the yeasts for *D. buzzatii*, the data have been subjected to analysis of variance using the factorial designs of:

4 sites × 6 yeasts × 2 sexes (expt 1)
2 days × 4 sites × 6 yeasts × 6 collections × 2 sexes (expts 2 and 3)

Significant effects for sites in all three experiments, and for days and collections in experiments 2 and 3 were not unexpected (Table 2). They simply reflect normal

variation in the distributions of flies in the locality, and their activity patterns from day to day and at different times of the day.

The numbers of each sex collected were not significantly different in any experiment but, more important, the yeasts × sex interaction and higher order interactions involving these also were not significant. Thus over the three experiments, there is no evidence for differences in the attractiveness of the yeasts for the two sexes of *D. buzzatii*.

Table 2. Analyses of variance of the distributions of *D. buzzatii* in each of experiments 1, 2 and 3
* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$

Source of variation	D.f.	Mean square	Source of variation	D.f.	Mean square (Expt 2)	Mean square (Expt 3)
	Experiment 1			*Experiments 2 and 3*		
Site (S)	3	59·06**	Day (D)	1	56·88*	4·52
Yeast (Y)	5	132·98***	Site (S)	3	32·47*	91·81***
Sex (X)	1	12·00	Yeast (Y)	5	227·81***	77·46***
S × Y	15	41·37**	Collection (C)	5	322·34***	10·79**
S × X	3	4·06	Sex (X)	1	8·75	4·17
Y × X	5	20·65	D × S	3	70·70***	7·28
			D × Y	5	32·47**	4·80
Error	15	9·44	D × C	5	202·94***	39·44***
			D × X	1	0·50	2·13
			S × Y	15	19·79*	12·19***
			S × C	15	18·79*	5·85
			S × X	3	16·69	0·53
			Y × C	25	35·22***	7·60***
			Y × X	5	3·32	4·42
			C × X	5	7·64	1·64
			D × S × Y	15	74·18***	6·30*
			D × S × C	15	40·85***	14·02***
			D × S × X	3	4·35	0·51
			D × Y × C	25	22·39***	4·79
			D × Y × X	5	7·80	0·66
			D × C × X	5	4·48	2·51
			S × Y × C	75	22·52***	5·42**
			S × Y × X	15	2·14	2·20
			S × C × X	15	3·38	2·29
			Y × C × X	25	2·23	2·75
			Error	280	9·76	3·51

However, there were highly significant differences ($P < 0{\cdot}001$) in all experiments in the numbers of *D. buzzatii* attracted to the different yeasts (Tables 2 and 3), although the rank order of attractiveness changed markedly from experiment to experiment. Although the yeast species were placed in a separate random order after each collection at each site, the patchy distribution of *Opuntia* rots is likely to result in a non-random distribution of flies in the area of attraction of each set of baits. Thus if flies were coming to the baits predominantly from one direction, more flies may be collected from the bait reached first, irrespective of the yeast in that bait. In this situation, confounding of yeast species and position where each yeast is located in the circle of baits would bias the measure of the true attractiveness

of each yeast. Because of the extreme non-orthogonality in the classification of yeasts × positions, it has not been possible to include both effects in a single analysis of variance.

However, the numbers of flies collected at each position (i.e. ignoring yeast species) have been analysed for experiments 2 and 3, and many of the interactions involving positions with collections, sites and days were significant. If there were no differences in attractiveness of the yeast species, and the numbers of flies collected at each bait were simply a function of non-random distribution of flies, then at each site, the rank order of positions (in terms of numbers of flies collected) should be approximately constant over collections and over days. The significant interactions for positions × collections, positions × days and positions × collections × days argue against numbers collected being a simple function of non-random fly distribution. Thus while position effects may contribute to the numbers of flies collected at each bait and cause some bias in the measured attractiveness of each yeast species, we conclude that differential attractiveness is the primary factor operating.

Table 3. Numbers of *D. buzzatii* attracted to each yeast

Values are average numbers of each sex at each site in experiment 1 and average numbers of each sex at each collection at each site on each day in experiments 2 and 3. Underlined means not significantly different by Tukey's *w* procedure

		Experiment 1			
Y1(a)	Y4	Y1(b)	Yx	YSc	Y2
3·00	7·50	7·75	8·12	12·87	14·25
		Experiment 2			
Y4	Yx	Y2	Y1(a)	YSc	Y1(b)
2·11	2·22	2·46	2·84	2·94	6·20
		Experiment 3			
Y2	YSc	Yx	Y1(a)	Y1(b)	Y4
1·17	1·55	2·17	2·34	2·38	3·78

When the data of experiments 2 and 3 were included in one analysis, with month (November *v.* April) as the extra variable, the months × yeasts interaction was highly significant ($P < 0{\cdot}001$). As the relative frequency of yeast species at 'Yarrawonga' shows quite marked seasonal variation (Barker, Phaff and Miranda, unpublished data), the measured relative attractiveness of the yeasts could be dependent on their frequency in the natural environment. These frequencies are given in Table 4, pooling for each experiment the results of yeast assays of samples taken at the time of the experiment and those taken one month previously. Samples taken at the time of each experiment were taken only from the four experimental sites, and equally from each. *Candida sonorensis* and *P. cactophila* are the commonest yeasts at 'Yarrawonga' and are relatively frequent in all months, while *Cryptococcus albidus* var. *albidus* occurs irregularly at low frequency. The distributions of yeast species at the times of the two experiments are significantly different ($\chi^2_{(16)} = 58{\cdot}64$,

$P < 0{\cdot}001$), and are also significantly different for the four yeast species used in the experiments ($\chi^2_{(3)} = 14{\cdot}31$, $P < 0{\cdot}01$).

While we have no direct evidence that prevalence of yeast species can affect the preferences exhibited by the flies, possible relationships between relative frequency and observed attractiveness should be noted. The most striking is for *P. cactophila* variety (Y4), which had lowest attractiveness and low frequency in experiment 2, highest attractiveness and much higher frequency in experiment 3. In addition, *P. cactophila* (Y2) had higher attractiveness and somewhat higher frequency in experiment 2 than in experiment 3. However, data were available only for the frequency of isolation of each species and not for relative cell concentrations in the rots, which may be more important in determining attractiveness. Further, any frequency-attractiveness relationship may be confounded by other yeast species in the natural environment. As shown in Table 4, many other species were isolated at each time, and if any of these have similar metabolic products, and hence similar attractiveness, to one or other of the tested yeast species, frequency-attractiveness relationships could be difficult to detect.

Table 4. Numbers of isolates and frequencies of wild yeasts at 'Yarrawonga' at the times of experiments 2 and 3

Yeast species	Number (frequency)	
	October–November, 1976 (Expt 2)	March–April, 1977 (Expt 3)
Candida sonorensis [Y1(a), Y1(b)]	19 (0·26)	21 (0·23)
Pichia cactophila (Y2)	15 (0·21)	16 (0·17)
Pichia cactophila variety (Y4)	1 (0·01)	13 (0·14)
Cryptococcus albidus var. *albidus* (Yx)	9 (0·13)	2 (0·02)
Candida mucilagina	4 (0·06)	3 (0·03)
Candida pulcherrima	1 (0·01)	— —
Pichia opuntiae var. *opuntiae*	5 (0·07)	2 (0·02)
Pichia amethionina var. *pachycereana*	1 (0·01)	6 (0·07)
Clavispora opuntiae	12 (0·17)	— —
Cryptococcus laurentii var. *laurentii*	2 (0·03)	2 (0·02)
Cryptococcus cereanus var.	1 (0·01)	2 (0·02)
Cryptococcus macerans	— —	2 (0·02)
Rhodotorula minuta var. *minuta*	— —	21 (0·23)
Rhodotorula minuta var. *texensis*	1 (0·01)	— —
Rhodotorula rubra	1 (0·01)	— —
Kloeckera japonica	— —	1 (0·01)
Kloeckera apiculata	— —	1 (0·01)
Total number of independent isolates	72	92

As noted previously, sufficient *D. aldrichi* for meaningful analysis were collected only in experiment 3. For factorial analysis of variance including both species, collections each day were pooled because of the smaller numbers collected of *D. aldrichi*, so that the data comprised numbers of flies of each sex of each species attracted to each yeast at each site on each day. As for *D. buzzatii* in all experiments, effects of sex, yeasts × sex and higher-order interactions involving these two variables were all non-significant. Effects of yeasts and yeasts × species, however, were both

Table 5. Summary of significant effects in the χ^2 analyses of the numbers of each allele and of each genotype at each of six loci in experiments 1, 2 and 3

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; † $P < 0{\cdot}10$

Source of variation	*Esterase-1*		*Esterase-2*			*β-N-Acetyl-hexosaminidase*		*Phosphoglucomutase*				*Aldehyde oxidase*		*Alcohol dehydrogenase-1*			
	Alleles		Alleles		Genotypes	Alleles		Alleles		Genotypes		Alleles		Alleles		Genotypes	
	d.f.	χ^2	d.f.	χ^2	d.f. χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2
Yeasts (Y)																	
Expt 1			15	28·31*	40 55·03†												
Expt 2	10	17·59†	15	23·73†	45 72·38**												
Expt 3						5	11·66*										
Day × Y																	
Expt 2														5	11·22†	10	23·43**
Site × Y																	
Expt 1												15	22·86†				
Expt 2	10	16·82†	45	63·74†										5	16·62**	10	18·08†
Expt 3	30	40·37†	45	64·44*													
Sex × Y																	
Expt 1			15	25·13*	40 54·47†			5	10·10†	5	10·34†						
Day × Site × Y																	
Expt 3										45	69·22*						

highly significant ($P < 0{\cdot}001$), the latter indicating significant differences between the species in their yeast preferences. The mean numbers of *D. buzzatii* attracted to each yeast are given in Table 3, while those for *D. aldrichi* (underlined means not significantly different by Tukey's *w* procedure) were:

YSc	Y1(b)	Yx	Y2	Y4	Y1(a)
3·00	3·06	3·13	4·13	5·00	5·12

Thus the two strains later identified as *C. sonorensis* were significantly different in attractiveness for *D. aldrichi*, as they were for *D. buzzatii* in experiment 2.

Table 6. Allele frequencies at the *Esterase-2* locus in flies attracted to each yeast in each experiment

Values in bold font are the highest ones for each allele in each experiment

No. of genomes ($2N$) and allele name	Yeast species					
	Y1(a)	Y1(b)	Y2	Y4	Yx	YSc
			Experiment 1			
$2N$	44	104	214	106	110	182
a	**0·52**	0·30	0·39	0·31	0·31	0·26
b	0·25	0·27	0·25	0·31	**0·33**	0·32
c	0·11	0·11	**0·14**	0·07	0·10	0·17
d	0·11	**0·33**	0·22	0·31	0·26	0·26
			Experiment 2			
$2N$	314	524	226	244	284	244
a	0·31	0·38	0·38	0·36	**0·42**	0·36
b	**0·43**	0·34	0·38	0·30	0·32	0·38
c	0·09	0·09	0·04	**0·10**	0·06	0·08
d	0·18	0·19	0·19	**0·24**	0·20	0·18
			Experiment 3			
$2N$	426	436	216	656	396	268
a	**0·32**	0·31	**0·32**	0·31	0·30	0·30
b	**0·34**	0·31	0·27	0·32	0·33	0·32
c	0·10	**0·14**	0·13	0·13	0·13	0·10
d	0·24	0·23	**0·28**	0·24	0·23	**0·28**

Allozyme Genotype–Yeast Relationships

In the χ^2 analyses, there were some significant differences for days, sites or sex for alleles or genotypes or both at one or more loci. However, the primary interest in these analyses is in whether there were significant differences in gene or genotpye frequencies in the flies attracted to different yeast species, or any significant interactions involving yeast species. A summary of the significant effects is given in Table 5, including those that were near to significance ($0{\cdot}05 < P < 0{\cdot}10$). While all loci have shown one or more effects involving yeast species that were significant or near to significance in one or more experiments, the evidence clearly is strongest for *Esterase-2*. For this locus, there were significant effects of yeast species on allele frequencies in experiment 1 and on genotype frequencies in experiment 2, and a significant site × yeast interaction for allele frequencies in experiment 3. However,

it must be noted that effects were not consistent over experiments. For example, allele frequencies for *Esterase-2* among flies attracted to each yeast in each experiment are given in Table 6.

Discussion

In view of previous studies demonstrating yeast preferences in a number of *Drosophila* species, the finding of such preferences for *D. buzzatii* and *D. aldrichi* was expected. However, it is clear that the preferences of *D. buzzatii* are not constant over time, and that the preferences of the two species differ.

There were some indications that the changes in preferences of *D. buzzatii* between experiments 2 and 3 could be related to the relative frequencies of the yeasts in natural rots at the experimental locality. That is, when a particular yeast species is more common in the natural environment, the flies may be 'conditioned' by utilization of that species and hence be more likely to be attracted to it. However, in a laboratory experiment designed to test whether flies grown on and stored as adults on a particular yeast species ('conditioned' by it) were affected in their subsequent attraction to this or other yeast species, no evidence for any conditioning effect was found (Barker *et al.* 1981). In that experiment, the flies used were 1–9 days old, presumably ample time for the development of any 'conditioning' effect. It is possible, however, that this laboratory result may not be relevant to natural populations, the difference being that in nature, flies are exposed from eclosion to a number of yeast species, and could develop a 'conditioned preference' for a particular species. Changes in the relative attractiveness of yeast species over 11 h in the laboratory experiments could be evidence for the development of such a conditioned preference. Nevertheless, while the reasons for the differences in relative attractiveness of the yeast species in the different experiments are not clear, the finding of such differences is important in showing that yeast preferences may not be fixed and species specific.

Within each experiment, interactions of yeasts with sites, days and collections generally were significant, i.e. the order of yeast attractiveness was not constant over time (collections over a 3-h period, or from one day to the next) or over space (sites). Again, site differences might be related to the relative frequency of each yeast at each site, but no such relationships could be discerned in the data. More useful information on this question might have been obtained had the yeast species in the crops of the flies attracted to each yeast been determined. However, given the changes in attractiveness of yeast species over collections within a 3-h period, it could be argued that such relationships should not be expected at the microenvironmental level, even though they may be important in relation to seasonal variation in yeast species frequency. The changes in relative attractiveness of the yeasts from one day to the next could be due to differential growth of the yeasts over the 24-h period, to cell autolysis and death, or to the formation of secondary metabolites, but these explanations would seem unlikely for changes over collections. With regard to these changes in attractiveness over collections, laboratory experiments (Barker *et al.* 1981) showed similar short-term variation for which no explanation could be given. However, in those experiments, there were contrasts in attractiveness of some of the yeast species for young flies and for mature females. Thus the variation in attractiveness over the collection period in the field could result from different activity patterns for flies of different ages, or from feeding and oviposition responses at different times.

While the different yeast preferences of *D. buzzatii* and *D. aldrichi* were not unexpected, and do indicate some degree of niche separation, it is unfortunate that these experiments were done before more detailed data on the relative frequencies of the yeast species were available. The responses of the two *Drosophila* species to *Cl. opuntiae* (high frequency at time of expt 2, absent at expt 3—Table 4) and *Rh. minuta* var. *minuta* (absent at time of expt 2, high frequency at expt 3) would be of particular interest, as the laboratory experiments showed for mature females that *D. buzzatii* had highest attraction to *Cl. opuntiae* but low attraction to *Rh. minuta* var. *minuta*, while *D. aldrichi* had highest attraction to *Rh. minuta* var. *minuta* and low attraction to *Cl. opuntiae*. While niche separation in terms of yeast species utilization may still be evolving in the Australian populations of *D. buzzatii* and *D. aldrichi*, the observed separation may be dependent simply on separate *Drosophila*–yeast evolution in the different regions of origin, *viz. D. buzzatii* in Argentina–Brazil (Carson and Wasserman 1965), *D. aldrichi* in Mexico–Texas (Patterson and Wheeler 1949). Unfortunately, no data are available on the yeast flora of *Opuntia* cactus rots in these regions. The high attraction of *D. aldrichi* to *Rh. minuta* var. *minuta* is particularly intriguing, as this yeast is not cactus-specific, and has been recovered from numerous habitats other than cacti.

Given our primary concern with the mechanisms maintaining genetic variation at isozyme loci in natural populations, the finding that different genotypes are differentially attracted to different yeast species suggests that nutritional variation may be one factor contributing to selection, particularly at the *Esterase-2* locus. The tissue distribution of esterases in *D. buzzatii* has not been examined, but *Esterase*-2 is α-specific and probably homologous with the C band of *D. aldrichi* found by Kambysellis *et al.* (1968) to be only in the gut. If this were true also for *D. buzzatii*, *Esterase*-2 may be a digestive enzyme. The physiological substrates of the esterases are not known, but different yeast species in monoculture on cactus produce different esters and different relative concentrations of different esters (East, unpublished data). If the preference for a yeast species by an *Esterase-2* genotype were related to the nutritional sufficiency of that yeast for that genotype, heterogeneity of yeast species, both spatially among rots and temporally, would contribute to the maintenance of polymorphism at the *Esterase-2* locus. Whether there is any relationship between preference for a particular yeast and its nutritional sufficiency has not yet been determined, and there is also the problem that the relative attractiveness of the yeast species for each genotype changed from one experiment to the next. Further, the evidence implicating selection at the *Esterase-2* locus is at best circumstantial, and we cannot rule out the possibility that selection is acting on linked genes, rather than the *Esterase-2* locus itself.

Clearly many uncertainties remain, but the indications that nutritional variation may contribute to selection, at least at the *Esterase-2* locus, are strong enough to encourage further experiments to enable a more critical analysis.

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