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

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Abstract

The attractiveness of yeast species isolated from rotting cladodes of *Opuntia inermis* for adults of the cactophilic species *D. buzzatii* and *D. aldrichi* was determined by giving the flies a multiple choice of yeast species and counting the numbers on each yeast at regular intervals throughout 1 day in each of five experiments. Consistent effects implying behavioural differences between sexes, between immature and mature flies, and between the two *Drosophila* species were found. For immature flies of each species, there were significant differences in the numbers of flies attracted to each yeast, but preferences were more marked for females than for males. Although there were no significant differences between the two *Drosophila* species in their yeast preferences, comparison of results for 1–3-day-old flies and mature mated females suggested possible differentiation between the species in yeast utilization by mature females, and possible larval–adult feeding niche separation. Behavioural responses contributing to observed effects, particularly differences between sexes, and between young and mature flies, are clearly complex and their basis remains to be elucidated.

Extra keywords: yeast preferences; feeding behaviour.

Introduction

According to Shehata and Mrak (1952), Dobzhansky in 1948 'expressed the opinion that yeasts are important in understanding some of the forces of natural selection to which the natural populations of *Drosophila* are exposed'. Subsequent studies of the relationships between yeasts and *Drosophila* have contributed greatly to the understanding of *Drosophila* ecology (Carson 1971) and have vindicated Dobzhansky's opinion.

Inferences relating to the action of natural selection have been largely in terms of species differences and differential adaptation. Thus Carson *et al.* (1956) explained their findings that conspecific larval and adult feeding sites were different while adults of different species shared the same feeding sites, as adaptations to reduce intraspecific competition in regions where suitable substrates are probably small and ephemeral. Similar results for other temperate *Drosophila* species were obtained in England by Begon (1973). On the other hand, da Cunha *et al.* (1957) showed that adults of tropical *Drosophila* species differed in the species of yeasts utilized and were able to discriminate among the yeasts. These results were interpreted as indicating the evolution of strategies to minimize interspecific competition where possible breeding and feeding substrates are both abundant and diverse. However, nutritional variation may contribute also to within-species selection, as da Cunha (1951) showed that the adaptive values of inversion karyotypes in *D. pseudoobscura* varied when different yeasts and bacteria were used as food sources.

More recently, there has been renewed interest in the analysis of yeast–*Drosophila* relationships, mainly in the work of Heed *et al.* (1976) and Starmer *et al.* (1976) on cactophilic *Drosophila* of the Sonoran Desert. These studies, which have the specific advantage that the breeding and feeding sites of the *Drosophila* species are well known (Fellows and Heed 1972; Heed 1978), have shown possible larval–adult niche separation in the yeast species utilized, and explained variation in habitat diversities of yeasts isolated from the crops of four *Drosophila* species partly by the feeding behaviour of the flies, partly by yeast composition of the host plants, and possibly by differential digestion of yeast species by adult flies.

Again primarily because of the known feeding and breeding site and apparent specificity to the cactus niche, we have chosen to study the cactophilic *Drosophila* in Australia (*D. buzzatii* and *D. aldrichi*), but with the main focus on the mechanisms maintaining genetic variation at isozyme loci in natural populations (Barker and Mulley 1976; Barker 1977; Mulley and Barker 1977; Mulley *et al.* 1979). Wagner (1944, 1949) showed that species of the *mulleri* subgroup (including *D. buzzatii* and *D. aldrichi*) differed in their ability to utilize for larval growth and development a number of yeast strains isolated from rotting *Opuntia* fruit. *D. buzzatii* and *D. aldrichi* were tested on five yeasts, of which two were nutritionally adequate for both, two did not support *D. aldrichi*, and one did not support *D. buzzatii*. As both species are commonly found breeding in the same *Opuntia* cladode rot in Australia (Mulley and Barker 1977), the species of yeasts present in the rots could contribute to selection operating both between and within these two *Drosophila* species. Preliminary assays of yeast species from rotting *Opuntia* cactus (Barker 1977) indicated a diversity of species among rots, both within and among localities (spatial heterogeneity) and among rots at different times within a locality (temporal heterogeneity). As environmental heterogeneity and consequent diversifying selection have been postulated as significant factors in the maintenance of genetic variation (see review of Hedrick *et al.* 1976), we suggest that the yeast–cactophilic *Drosophila* system provides an excellent opportunity to test the hypothesis.

The experiments reported here used yeast species isolated from rotting *Opuntia* cladodes, and relate only to the choice of yeast species by adult flies under laboratory conditions.

Materials and Methods

Strains of Flies and Culture Conditions

The strains of *D. buzzatii* and *D. aldrichi* used in these experiments were from collections made at 'Yarrawonga', Hunter Valley, New South Wales, [locality 5 of Barker and Mulley (1976)].

D. buzzatii for experiment 1 were derived from 75 isofemale lines collected in March–April 1975, and maintained separately in vial cultures for four generations. Each line then contributed equal numbers of adults to initiate six population cages, and after six generations, equal numbers of adults from each cage were mixed and set up in vials. Experimental flies were 1–3-day-old virgin progeny from these vials.

D. buzzatii for experiments 2–5 were from new cages set up in April 1977, which were derived from 96 isofemale lines collected during June–December 1976. These lines, maintained as vial cultures for 4–12 generations, were crossed in pairs (1×2, 2×3, $3 \times 4 \ldots 96 \times 1$) and three males and three females (collected as virgins) from each cross were used to initiate each of six cages. Flies for experiment 2 were the progeny of adults sampled from one cage approximately six to seven generations after cage initiation, and those for experiments 3–5 were progeny of adults sampled from another cage approximately 29–30 generations after cage initiation.

D. aldrichi collected at 'Yarrawonga' during April–December 1977 were not maintained as isofemale lines but as eight separate mass-culture stocks. After 8–17 generations, interstock crosses were set up and 20 pairs of virgin progeny from each of eight crosses were the initial populations for each of two cages. Flies for experiments 3–5 were progeny of adults sampled equally from both cages approximately two generations after cage initiation.

D. buzzatii were maintained throughout (in both cages and vials) on an autoclaved agar–sucrose–yeast (*Saccharomyces cerevisiae*) medium comprising 50 g agar, 180 g sucrose, 300 g yeast, 3200 ml water and 3·6 g methyl-*p*-hydroxybenzoate. In experiments 3 and 5, experimental flies were stored for a period on an agar–sucrose medium which was as above except without yeast. *D. aldrichi* do not culture well on the agar–sucrose–yeast medium, but Richardson and Kambysellis (1968) found a cactus-supplemented banana food to be satisfactory. We have used a cactus-supplemented modification of our yeast (*S. cerevisiae*)-fortified medium (Claringbold and Barker 1961), comprising 4 g agar, 75 g yeast, 40 ml treacle, 40 g cornmeal, 175 ml cactus slurry, 500 ml water, 0·5 g methyl-*p*-hydroxybenzoate and 2·5 ml propionic acid. Cactus slurry was prepared by cutting fresh *Opuntia inermis* cladodes into pieces approximately 3 by 3 cm, autoclaving and thoroughly blending.

Experimental flies were raised in 7·6 by 2·5 cm vials containing 7 ml of the appropriate medium, using five pairs of parents per vial which were discarded after 7 days.

Yeast Species and Yeast Production

A number of yeast strains isolated from rotting cladodes of *O. inermis* at 'Yarrawonga' were identified by colony or spore morphology or both, and five of these were used in experiments 1 and 2. Results of experiment 1 were discussed briefly by Barker (1977), in which preliminary identifications of these yeast species were given. However, one strain, identified there as *Pichia* sp. (code No. Y6), was not in fact used in experiments 1 and 2. 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 here are given to correspond to the yeast species used in experiments 3–5):

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*) were initially 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 they were differentially attractive to *D. buzzatii* in experiment 1 (see Results, Table 2) and as the identifications done by Professor Phaff and Ms Miranda were done after experiment 2, it seems most likely that a mistake was made in strain maintenance after experiment 2 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 about 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 (H. J. Phaff and M. 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.

All yeasts were cultured at 25°C. For experiments 1 and 2, one colony of each strain was placed in 10 ml nutrient broth (Wagner 1949). After 2 days, 290 ml nutrient broth was added and growth continued for a further 3 days with constant agitation. The suspension was then centrifuged, the yeast paste transferred to 1500 ml nutrient broth and again left for 3 days with constant agitation. Following centrifugation, each yeast was collected as a thick paste, with yields in experiment 2 ranging from 15·5 g for Y1(*b*) to 22·5 g for Y4. For use in the experiments, the yeast paste was suspended in sterile distilled water (1 g paste:1 ml water in expt 1; 1:2 in expt 2).

The nine yeast species used in experiments 3–5 were taken from 376 yeasts isolated from 279 rotting cladodes of *O. inermis* collected at 'Yarrawonga' from October 1976 to December 1977. These isolates were identified by Professor Phaff and Ms Miranda, and the 10 most common species were as follows (in order of decreasing frequency in the collections):

Code No.	Species
Y1	*Candida sonorensis* Miller *et al.*
Y2	*Pichia cactophila* Starmer *et al.*
Y3	*Lodderomyces opuntiae* Phaff *et al.*
Y4	*Pichia cactophila* variety
Y5	*Rhodotorula minuta* (Saito) Harrison var. *minuta*
Y6	*Candida mucilagina* Phaff *et al.*
Y7	*Pichia opuntiae* Starmer *et al.* var. *opuntiae*
Y8	*Cryptococcus albidus* (Saito) Skinner var. *albidus*
Y9	*Pichia amethionina* Starmer *et al.* var. *pachycereana*
Y10	*Cryptococcus cereanus* Phaff *et al.* var.

Of these 10 most common yeast species, Y8 (Yx for expts 1 and 2) was not used in the experiments, as it had originally been misclassified as two different varieties.

Yeasts for experiments 3, 4 and 5 were prepared by taking one or two colonies of each species and spreading on two Wagner (1949) nutrient agar slopes. After growth at 25°C for 2 days, the yeast on the slopes was collected and inoculated into 300 ml nutrient broth, which was left for 2 days at 25°C with air continuously bubbled through the broth. Following centrifugation, the yeast paste was collected and a cell count of each species done using serial dilution and a haemocytometer. *Rh. minuta* var. *minuta* had the lowest cell concentration and was diluted at the rate of 1 g paste to 1 ml sterile distilled water. All other yeasts were diluted with appropriate volumes of sterile distilled water to bring them to the same cell concentration.

Experimental Techniques

All experiments were done under constant illumination at 25 ± 0·5°C, 65–70% relative humidity in small plastic population cages. In experiments 1 and 2, the cages were 20 by 14 by 7·5 cm with six holes for attachment of cage jars in the base, while in experiments 3, 4 and 5, cages 22 by 22 by 7·5 cm with nine holes in the base were used. Corks (3·8 cm smaller diameter) placed in the base holes of the cages had a 3·4 cm diameter, 4 mm deep depression cut in the smaller end, and carried agar discs on which one or other yeast species was smeared. Discs (3·3 cm diam., 3–4 mm thick) were cut from a slab of 1·5% agar (experiments 1 and 2) or 1·5% agar, 20% cactus slurry (experiments 3–5), and smeared with 0·3 ml yeast suspension. The cactus slurry was prepared as for the *D. aldrichi* medium, except that fibrous material was strained off after blending.

In experiments 1 and 2, five discs (each with a different yeast species) and one empty cork were assigned at random to the six positions in the base of each cage. In experiments 3, 4 and 5, the nine discs put in each cage were assigned at random to the nine positions.

In experiment 1, yeast discs were prepared at 0830 h on the morning of the experiment and placed in the cages. Each cage was covered by a sheet of clear plastic and left at 25°C for at least 1 h. The appropriate flies then were added to each cage without anaesthetization, and all cages were set up by 1030 h. The cages then were not disturbed in any way until completion of scoring. The numbers of flies on each disc in each cage were counted every 0·5 h from 1215 to 1615 h to give nine replicates, with discs within cages and cages scored in the same order each time.

In experiments 2–5, yeast discs were prepared the evening before each experimental run and placed in the cages, the top of each cage was covered by a sheet of clear plastic, and the complete cages stored overnight at 4°C. At 0530 h the next day, the cages were placed at 25°C, and after about 20 min flies were added to the cages without anaesthetization. All cages were set up by 0630 h, and the numbers of flies on each disc in each cage were counted every 1 h from 0730 to 1830 h. Other procedures were as for experiment 1.

Experimental Design

Except for experiment 2, all experiments were designed to determine the preferences of adult flies for different yeasts, using flies of different ages, sexes separate or mixed, and with or without prior yeast starvation. The design and a summarized description of each experiment is given in Table 1.

Experiment 1

In experiment 1, three cages were set up with males only and three with females only. With nine observation times for each cage, the design was (two sexes with three replicate cages) × five yeasts × nine times.

Experiment 2

Experiment 2 was designed to determine whether the yeast species present during the larval and pupal stages and in the first few days of adult life had any effect on the yeast preferences of adult flies.

Experimental flies were reared on autoclaved agar–sucrose–yeast (*Saccharomyces cerevisiae*) medium, to the surface of which was added (after cooling of the medium) 0·2 ml of 1:2 yeast suspension of one of the five yeasts tested. The vials were stored at 4°C for a maximum of 2 days. Flies emerging from these vials were collected daily as virgins and stored at 25°C in medium vials with the same live yeast as was used for their development (15 progeny per vial, sexes separate). Progeny collection continued over 8 days, so adults were 1–9 days old when used for the experiment.

Table 1. Summary of the design and the flies used in each experiment

Design treatments

Expt No.	No. of species	No. of days	No. of sexes	No. of replicate cages	No. of rearing yeasts	No. of attractant yeasts	No. of observation times
1			2	3		5	9
2		3	2		5	5	12
3	2		2	5		9	12
4	2			5		9	12
5	2			2(*b*), 4(*a*)[A]		9	12

Flies used

Expt No.	No. of flies per cage	Age of flies (days)	Sexes	Pretreatment
1	150	1–3	♂ and virgin ♀ separate	
2	100	1–9	♂ and virgin ♀ separate	
3	200	1–3	♂ and virgin ♀ separate	20 h yeast starvation
4	200	1–3	♂ and ♀ together	
5	200	4–6	Mated ♀ only	20 h yeast starvation

[A] *b*, *D. buzzatii*; *a*, *D. aldrichi*.

The experiment was replicated over 3 consecutive days, with the flies required for each day produced separately by the above procedures. On each day, 10 experimental cages were initiated. Five cages had males only, five females only, with one cage for each sex containing adults raised and stored on one of the yeast species. The design of experiment 2 was thus 3 days × two sexes × five rearing yeasts × five attractant yeasts × 12 scoring times.

Flies are attracted to the yeasted discs presumably to feed or, for females, to oviposit. Therefore an assay of the crop contents of individual flies should give an indication of the extent to which individual yeasts were ingested. After the last observation time, 98 flies from cage 9 (day 2, females raised on Yx) were so assayed. These flies were etherized, placed in absolute alcohol for 1 min as a surface sterilant, washed in distilled water and placed on plates containing Wagner's (1949) nutrient medium. They were then squashed and the crop contents plated by dilution streaking. After incubation at 25°C for 2 days, each plate was scored for presence or absence of each yeast species, as identified by colony and spore morphology.

Experiment 3

Experiment 3 included *D. aldrichi* as well as *D. buzzatii*. Experimental flies, progeny of adults sampled from the stock cages, were collected as virgins over 2 days. Those collected on the first day were stored (25 per vial) on agar–sucrose–yeast medium. Those collected on the second day were stored on agar–sucrose medium, and those from the first day were transferred to this medium at this time. The experiment was run the next day, so experimental flies were 1–3 days old when added to the cages, and had been stored for 20 h on medium containing no yeast (either live or dead).

Twenty cages were set up in a design of (two species × two sexes with five replicate cages) × nine yeasts × 12 scoring times.

Experiment 4

Experimental flies in experiment 4 were produced as in experiment 3, but on emergence were stored (sexes separate) on agar–sucrose–yeast medium only. Again, flies were 1–3 days old when the cages were set up. Ten cages were used, five for *D. buzzatii*, five for *D. aldrichi*, with each cage containing 100 males and 100 females, and a design of (two species with five replicate cages) × nine yeasts × 12 scoring times.

Experiment 5

Procedures in experiment 5 were as for experiment 4, except that experimental flies were stored from emergence at five pairs per vial until they were 3–5 days old. The males then were discarded and the females stored (25 per vial) on agar–sucrose medium for 20 h before cage initiation. Two cages were set up with *D. buzzatii* and four with *D. aldrichi*, each cage with 200 females. Except for the unequal replication of cages within species, the design was as for experiment 4.

After the last count of flies on the yeast discs at 1830 h, the yeast discs were removed from the cages and the numbers of eggs laid on each counted.

Table 2. Significance of differences among treatments in experiment 1, as determined by Tukey's *w* procedure

Treatment means underlined were not significantly different

Yeasts									
Yeast Code No.				Y1(*b*)	Y4	Yx	Y1(*a*)	Y2	
Mean No. of flies				0·91	2·63	5·65	6·63	15·50	
Times									
Time No.	1	2	3	4	5	6	7	9	8
Mean No. of flies	1·93	3·17	4·27	5·47	6·67	7·83	8·20	9·07	9·77

Results

Experiment 1

Taking the number of flies on each yeast disc at each counting time as the unit of observation, analysis of variance showed significant effects for yeasts ($P < 0{\cdot}001$), times ($P < 0{\cdot}001$) and yeasts × times ($P < 0{\cdot}05$). Y2 clearly was most attractive and Y1(*b*) least, the latter being significantly different from Y1(*a*) (identified later as the same species, but see Materials and Methods), Yx and Y2 (Table 2). The significant time effect was due to a general increase in attractiveness over the nine observation times. However, the yeasts × times interaction was significant because differences among times were not significant for Y1(*b*), Y4 and Yx.

[illegible] added to the cages without anaesthetization. All cages were set up by 0630 h, and

Experiment 2

The design of this experiment was essentially similar to studies on the effect of medium conditioning in *Drosophila* (Dolan and Robertson 1975) in that we are asking whether flies grown on a particular yeast species ('conditioned' by it) are affected in their subsequent preferences for (or attraction to) this or other yeast species. That is, we are particularly concerned to determine whether there is any self-conditioning (where flies are preferentially attracted to the yeast on which they have developed) or any interaction between yeasts as attractors and yeasts as conditioners. For analysis, the unit of observation was taken as the total number of flies counted on each disc in each cage (sum of counts over the 12 times), the 3 days were treated as replicates, and males and females were treated separately. Table 3 gives the average numbers (over replicates) of males and females counted on each yeast disc.

Table 3. Average numbers (over three replicates) of flies counted on each attractor yeast when raised on each of the same yeasts as conditioners

Yeast as conditioner	Average No. of flies on attractor yeast					Average No. of flies for each conditioner yeast
	Y1(*a*)	Y1(*b*)	Y2	Y4	Yx	
			Males			
Y1(*a*)	9·33	7·00	10·33	7·67	8·67	8·60
Y1(*b*)	14·33	9·00	8·00	5·67	6·67	8·73
Y2	6·00	5·00	16·00	12·33	8·67	9·60
Y4	7·67	2·67	13·00	5·67	5·33	6·87
Yx	11·67	6·00	13·00	8·67	6·33	9·13
Average No. of flies for each attractor yeast	9·80	5·93	12·07	8·00	7·13	
			Females			
Y1(*a*)	23·33	10·33	51·00	17·00	40·00	28·33
Y1(*b*)	18·67	15·00	63·00	18·67	25·33	28·13
Y2	12·33	27·00	53·67	32·33	32·67	31·60
Y4	15·33	16·00	48·33	27·33	10·00	23·40
Yx	15·33	18·33	35·67	16·67	29·00	23·00
Average No. of flies for each attractor yeast	17·00	17·33	50·33	22·40	27·40	

The model and method of analysis were the same as those of Dolan and Robertson (1975), except that we had three replicates in each of the 25 cells for each sex, and the between-replicate variance was used as error variance. Yeasts as attractors were significantly different for both sexes (males, $P < 0{\cdot}01$; females, $P < 0{\cdot}001$), but there was no evidence for any conditioning effect.

Thus, further analyses were done treating the cages (i.e. flies raised on the different yeasts) as replicates, with a design of (3 days × two sexes with five replicate cages) × five yeasts × 12 times (Table 4*a*). As expected from the previous analysis, the effect of yeasts was significant, with Y2 significantly more attractive than the other four

which were not different from each other (Table 5). Significantly more females than males were attracted to the yeasts (average numbers: 2·56 *v.* 0·72), but in contrast to the first analysis, there were no significant differences among yeasts for males,

Table 4. Analyses of variance of the data of experiment 2 for 12 observation times (*a*) and four periods (*b*)

Those parts of analysis (*b*) which have identical *F*-tests to analysis (*a*) were omitted. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$

Source of variation	d.f.	m.s.	*F*	Source of variation	d.f.	m.s.	*F*
(*a*)				(*a*) *contd*			
Days	2	8·28	<1·00	Yeasts × times	44	4·05	1·38*
Sex	1	1536·43	98·11***	Days × yeasts × times	88	2·96	1·01
Days × sex	2	4·61	<1·00	Sex × yeasts × times	44	5·21	1·77*
Cages (days × sex)	24	15·66		Days × sex × yeasts × times	88	3·97	1·35*
Yeasts	4	203·56	15·24***	Cages (days × sex) × yeasts × times	1056	2·94	
Days × yeasts	8	24·34	1·82				
Sex × yeasts	4	130·62	9·78***				
Days × sex × yeasts	8	40·76	3·05**	(*b*)			
Cages (days × sex) × yeasts	96	13·36		Periods	3	985·25	40·75***
				Days × periods	6	38·58	1·60
				Sex × periods	3	395·47	16·36***
Times	11	97·17	27·71***	Days × sex × periods	6	28·18	1·17
Days × times	22	4·83	1·38	Cages (days × sex) × periods	72	24·18	
Sex × times	11	38·59	11·00***				
Days × sex × times	22	4·15	1·18				
Cages (days × sex) × times	264	3·51		Yeasts × periods	12	33·23	1·93
				Days × yeasts × periods	24	22·67	1·32
				Sex × yeasts × periods	12	40·69	2·36*
				Days × sex × yeasts × periods	24	28·47	1·65
				Cages (days × sex) × yeasts × periods	288	17·23	

Table 5. Significance of differences among treatments in experiment 2, as determined by Tukey's *w* procedure

Treatment means underlined were not significantly different

Yeasts					
Yeast Code No.	1(*b*)	1(*a*)	4	x	2
Mean No. of flies	1·07	1·22	1·43	1·54	2·94
Periods					
Period No.	1	2	3	4	
Mean No. of flies	1·73	4·04	6·43	7·47	
Sex × Periods					
Period No.	1	2	3	4	
Sex					
Males	1·12	1·59	2·63	3·25	
Females	2·35	6·49	10·24	11·68	

although there were for females (i.e. significant sex × yeasts interaction). The days × sex × yeasts interaction was significant in that there were no significant

differences among days × yeasts for males, while for females, although Y2 was most attractive every day, there were no significant differences among yeasts on day 3.

The numbers of flies attracted to the yeast discs generally increased through the 12 observation times, and the sex × times interaction was significant because there were significant differences among times for females, but not for males. There were no significant differences among yeasts in times 1–4, but Y2 was significantly more attractive than most of the other four yeasts in times 5–12. Yeasts other than Y2 showed little difference in attractiveness over time, except for some increase at the last three times.

Table 6. Distribution of yeast species isolated from 98 females from cage 9 (day 2) at the end of the observations in experiment 2 (*a*) and comparison of percentage of flies with each yeast in the crop with percentage in cage 9 (day 2) attracted to yeasted discs (*b*)

(*a*) No. of yeast species found in each fly	Yeast species	No. of flies	Percentage of flies
0	—	3	3·06
1	Y1(*b*)	1	1·02
	Y2	30	30·61
	Y4	16	16·33
2	Y1(*a*), Y2	1	1·02
	Y1(*a*), Y4	2	2·04
	Y1(*b*), Y2	12	12·24
	Y1(*b*), Y4	8	8·16
	Y2, Y4	16	16·33
3	Y1(*b*), Y2, Y4	9	9·18

(*b*) Yeast species	No. of flies with each yeast in crop (%)	No. of flies attracted to each yeast disc (%) Times 1–12 (n = 198)	Time 12 only (n = 31)	Times 11 and 12 only (n = 57)
Y1(*a*)	2·0 (3)	9·1 (18)	3·2 (1)	5·3 (3)
Y1(*b*)	19·7 (30)	0·5 (1)	3·2 (1)	1·8 (1)
Y2	44·7 (68)	66·7 (132)	74·2 (23)	71·9 (41)
Y4	33·6 (51)	11·6 (23)	16·1 (5)	14·0 (8)
Yx	0·0 (0)	12·1 (24)	3·2 (1)	7·0 (4)

Because effects involving times are difficult to interpret or illustrate or both with 12 observation times, the data were grouped into periods of times 1–3, 4–6, 7–9 and 10–12, and reanalysed (Table 4*b*). Two of the interactions involving times were not significant for periods, but there were still highly significant effects of periods and sex × periods (Table 5). Analysis of the significant sex × yeasts × periods interaction showed for males no significant differences among yeasts × periods. However, for females, although there were no significant differences among yeasts in period 1, Y2 was significantly more attractive than the other four yeasts (which were not different) in periods 2, 3 and 4.

The results of the yeast assay in the crop contents of 98 females from cage 9 are summarized in Table 6*a*. No yeasts were isolated from three flies, one from 47 flies,

two from 39 flies and three from nine flies, but one yeast (Yx) was not found in any fly. If flies are attracted to the yeast discs primarily to feed, the percentage of the flies counted on each yeast should be similar to the percentage with that yeast in the crop. As King and Wilson (1955) found that the time required for food passage through the digestive tract of *D. melanogaster* was 4–5 h, comparisons of the percentage of flies carrying each yeast in the crop with the percentage attracted to each yeast disc (Table 6*b*) were made for the later observation times. However, the latter percentage did not change dramatically when cumulated over times from time 12 back to time 1, and the cumulative percentages over the last five or six observation times were very similar to that for all times. In any case, differences in the distributions of flies

Table 7. Analyses of variance of the data of experiment 3, for 12 observation times (*a*) and four periods (*b*)

Those parts of analysis (*b*) which have identical *F*-tests to analysis (*a*) have been omitted. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$

Source of variation	d.f.	m.s.	*F*	Source of variation	d.f.	m.s.	*F*
(*a*)				(*a*) *contd*			
Species	1	2300·20	58·06***	Yeasts × times	88	8·33	1·65**
Sex	1	5481·70	138·35***	Species × yeasts × times	88	6·97	1·38*
Species × sex	1	1377·60	34·77***	Sex × yeasts × times	88	6·60	1·31*
Cages (species × sex)	16	39·62		Species × sex × yeasts × times	88	6·16	1·22
Yeasts	8	296·61	4·56***	Cages (species × sex) × yeasts × times	1408	5·04	
Species × yeasts	8	50·46	< 1·00				
Sex × yeasts	8	136·58	2·10*				
Species × sex × yeasts	8	40·51	< 1·00	(*b*)			
Cages (species × sex) × yeasts	128	65·09		Periods	3	212·05	9·30***
				Species × periods	3	132·38	5·81**
				Sex × periods	3	219·97	9·65***
Times	11	48·71	10·96***	Species × sex × periods	3	45·65	2·00
Species × times	11	32·92	7·40***	Cages (species × sex) × periods	48	22·80	
Sex × times	11	27·70	6·23***				
Species × sex × times	11	12·57	2·83**	Yeasts × periods	24	56·18	1·99**
Cages (species × sex) × times	176	4·45		Species × yeasts × periods	24	38·62	1·37
				Sex × yeasts × periods	24	36·69	1·30
				Species × sex × yeasts × periods	24	32·22	1·14
				Cages (species × sex) × yeasts × periods	384	28·21	

attracted to the discs and of crop contents are so marked that any variation in time of ingestion of particular yeasts is not likely to have biased the results. The yeast most attractive to the flies (Y2) was also most common in the crop, but there were marked discrepancies for other yeasts, particularly Y1(*b*) and Y4. Over the 12 observation times, a total of 23 flies was counted on Y4 and one on Y1(*b*), yet of the 98 females assayed (there were 100 in the cage for the observation period), 51 had Y4 in the crop and 30 had Y1(*b*). It would seem that flies visited these yeast discs to feed, but did not remain on the disc for very long. In contrast, 18 flies were observed on Y1(*a*) and 24 on Yx, but only three had Y1(*a*) in the crop and none had Yx. Flies

visiting these two yeasts presumably remained on the disc for a longer time, but did not feed. Hence, even though these females were 1–9-day-old virgins, the attraction may have been to a mating site or an 'oviposition' rather than a feeding response. The differential responses to Y1(*a*) and Y1(*b*) in relative attractiveness and crop contents emphasises that these strains must have been different at the time the experiment was done, even though they were both later identified as *C. sonorensis*.

Experiment 3

Again the number of flies on each yeast disc at each counting time was taken as the unit of observation, and the experiment was first analysed as (two species × two sexes with five replicate cages) × nine yeasts × 12 times (Table 7*a*). Significantly more *D. buzzatii* than *D. aldrichi* were attracted to the discs (4·84 *v.* 2·77), and significantly more females than males (5·40 *v.* 2·21), with the species × sex interaction also significant, as the sex difference was greater for *D. buzzatii* (2·45 males and 7·23 females) than for *D. aldrichi* (1·98 and 3·57). Although the main effect for yeasts was significant, the significant sex × yeasts interaction is more interesting, in that there were no significant differences among yeasts for males, but there were for females (Table 8).

Table 8. Significance of differences among treatments in experiment 3 as determined by Tukey's *w* procedure

Treatment means underlined were not significantly different

Sex × yeast									
Yeast Code No.	5	1	9	4	3	10	7	6	2
Male	1·50	1·88	1·96	2·20	2·33	2·33	2·34	2·36	3·03
Yeast Code No.	5	1	6	9	4	7	10	3	2
Female	2·52	3·40	3·99	5·17	5·52	6·08	6·43	7·23	8·28

Species × periods				
Period	1	4	3	2
D. buzzatii	12·08	14·76	15·01	16·21
Period	1	3	4	2
D. aldrichi	7·54	7·62	8·77	9·36

Sex × periods				
Period	3	1	2	4
Male	6·01	6·02	6·63	7·89
Period	1	4	2	3
Female	13·60	15·63	17·73	17·82

The significant effects for times and yeasts × times and interactions involving them are more readily interpreted when the data are grouped into periods, as was done for experiment 2 (Table 7*b*). Three of the three-way interactions involving times were no longer significant when the data were grouped into periods. Again, while there were significant differences among periods, the species × periods and sex × periods interactions are more interesting (Table 8). *D. buzzatii* showed lower attraction to the yeast discs in the first period than in later ones, while there were no differences for *D. aldrichi*. Similarly, there were no differences among periods for

males, and females showed lower attraction in the first and last period than in periods 2 and 3.

The significant yeasts × periods interaction is particularly interesting in showing that the reaction of the flies to different yeast species changed over time. Not all yeasts were least attractive in period 1. Some (e.g. Y9) were very constant over periods, others steadily increased, and yet others increased and then decreased. Thus, the order of attractiveness of the different yeast species changed from period to period. In general, the most and least attractive species were remarkably constant: Y2 was most attractive in periods 1–3, and ranked fourth in period 4, while Y1 and Y5 were consistently least attractive. On the other hand, Y3, Y7 and Y10 increased in attractiveness over the four periods, while Y4 steadily decreased.

Although the yeast species were placed in a separate random order in each of the 20 cages, there is the possibility that flies may prefer the corners rather than the edges of the square cage, or vice versa, or may be more likely to avoid the central position in the cage. Thus, effects of positions *per se* could confound real attraction to the different yeasts. It has not been possible to include both yeasts and positions in the one analysis of variance, but the numbers of flies on each disc at each position (i.e. ignoring yeast species) have been analysed. Only the positions × times interaction was significant ($P < 0{\cdot}05$), and as there was no obvious pattern in this interaction, we conclude that position effects *per se* were not important.

Table 9. Analyses of variance of the data of experiment 4, for 12 observation times (*a*) and four periods (*b*)

Those parts of analysis (*b*) which have identical *F*-tests to analysis (*a*) have been omitted. $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}001$

Source of variation	d.f.	m.s.	*F*	Source of variation	d.f.	m.s.	*F*
(*a*)				(*a*) *contd*			
Species	1	634·80	6·10*	Yeasts × times	88	7·91	1·49*
Cages (species)	8	104·00		Species × yeasts × times	88	5·91	1·11
				Cages (species) × yeasts × times	704	5·32	
Yeasts	8	127·10	3·44**				
Species × yeasts	8	47·29	1·28				
Cages(species) × yeasts	64	36·99		(*b*)			
				Periods	3	265·20	7·14**
Times	11	63·08	11·51***	Species × periods	3	340·50	9·16***
Species × times	11	38·41	7·01***	Cages (species) × periods	24	37·16	
Cages (species) × times	88	5·48					
				Yeasts × periods	24	52·64	1·77*
				Species × yeasts × periods	24	34·27	1·15
				Cages (species) × yeasts × periods	192	29·77	

Experiment 4

In this experiment, each cage contained equal numbers of 1–3-day-old virgin males and females, and the aim was to determine if there were any differential effects as compared with experiment 3 in which males and females were observed separately.

The basic observations were as in previous experiments, and analyses of variance are given in Table 9. Again, significantly more *D. buzzatii* (4·17) were attracted to

the yeasts than *D. aldrichi* (2·64), and these mean numbers are similar to those of experiment 3 (4·84 and 2·77 respectively). Again, the main effect for yeasts was significant, although the rank order of yeast attractiveness was somewhat different and there were fewer significant differences among yeasts as compared with experiment 3. The overall rank order in experiment 3 was the same as that shown for females in Table 8 (sex × yeast); the order in this experiment is given in Table 10. The smaller average number of flies per disc and the poorer discrimination among yeasts in this experiment than in experiment 3 may have resulted from the experimental flies not being stored on a yeast-free medium before the beginning of this experiment. The significant effects of times, species × times and yeasts × times are more readily interpreted in terms of periods (Table 9*b*), and comparisons among treatment means for species × periods are given in Table 10. These results are qualitatively similar to those in experiment 3, with no differences among periods for *D. aldrichi* and the attractiveness of the yeasts generally increasing over time for *D. buzzatii*.

Table 10. Significance of differences among treatments in experiment 4 as determined by Tukey's *w* procedure

Treatment means underlined were not significantly different

Yeast									
Yeast Code No.	5	6	1	10	9	4	2	7	3
Mean No. of flies	2·23	2·50	2·72	2·83	3·13	3·29	4·21	4·46	5·31
Species × periods									
Period			1	3	2	4			
D. buzzatii			8·58	11·98	12·67	16·87			
Period			3	4	1	2			
D. aldrichi			6·58	7·27	8·09	9·76			

The significant yeasts × periods interaction again indicated that the order of attractiveness of the different yeast species changed from period to period. However, the patterns of change were different from those of experiment 3. Y2, which had been consistently highly attractive in experiment 3, was again so in periods 1 and 4 but was less attractive in periods 2 and 3. Y3, Y7 and Y10 had steadily increased in attractiveness in experiment 3, but here Y3 and Y7 were highly attractive throughout, while Y10 generally decreased to be the least attractive in period 4.

Analysis of the effects of positions showed only a significant species × positions interaction ($P < 0{\cdot}05$). *D. buzzatii* showed a preference for the corners rather than the edges (mean number of flies per disc over the 12 times of 4·81 and 3·86 respectively), while *D. aldrichi* tended to the reverse preference (2·42 and 2·79). The magnitudes of the effects, however, are small and not likely to bias estimates of yeast preferences, although the error variance would be increased and the significance of discrimination among yeast species would be reduced.

Experiment 5

In this experiment, the flies were females only, older than in previous experiments, and had had the opportunity to be inseminated prior to the experimental observations.

The basic observations and analysis of variance (Table 11) were as for previous experiments. Again *D. buzzatii* were more attracted to the yeasts than *D. aldrichi*, although the difference in this experiment (averages of 6·56 and 5·27) was less than for the females in experiment 3 (7·23 and 3·57). Effects of times and species × times were significant, but were different from those of previous experiments. Whereas there were no differences among times for *D. aldrichi* in experiments 3 and 4 and *D. buzzatii* tended to show greater attraction to the yeasts in later times, *D. aldrichi* in this experiment showed a steady, significant increase over time, while *D. buzzatii* showed highest attraction in times 2–6 inclusive.

Table 11. Analysis of variance of the data of experiment 5

$*P < 0{\cdot}05$; $***P < 0{\cdot}001$

Source of variation	d.f.	m.s.	*F*
Species	1	238·50	17·30*
Cages (species)	4	13·79	
Yeasts	8	22·86	<1·00
Species × yeasts	8	78·00	1·01
Cages (species) × yeasts	32	77·18	
Times	11	99·58	8·14***
Species × times	11	110·90	9·06***
Cages (species) × times	44	12·24	
Yeasts × times	88	8·87	<1·00
Species × yeasts × times	88	11·68	
Cages (species) × yeasts × times	352	11·80	<1·00

The most striking result of this experiment, however, was the lack of significance of differences among yeasts, or of any interaction involving yeasts. As the flies used were mature, potentially inseminated females, responses to the yeast species would have been determined by both feeding and oviposition behaviours. To the extent that different yeast species are preferred for these two purposes, which would be confounded in the observations, differences in attractiveness among yeast species would be obscured.

Table 12. Means ± s.e. for total number of flies per yeast, number of eggs per yeast and ratio of eggs per fly for each species in experiment 5

Species	No. flies per yeast	No. eggs per yeast	Ratio
D. buzzatii	78·7 ± 6·05	253·6 ± 46·06	3·21 ± 0·43
D. aldrichi	63·2 ± 4·78	419·6 ± 55·12	6·63 ± 0·79

Effects of positions in the cage were analysed as in the previous experiments, but none were significant.

In an attempt to measure oviposition responses, the number of eggs on each yeast disc was counted at the end of the observations. Analyses of variance of the number of eggs per yeast disc and the ratio of number of eggs per yeast disc to the total number of flies counted per yeast disc showed the only significant effect ($P < 0{\cdot}05$)

as a species difference in the ratio. The means of each variable for each species (Table 12) show that although the number of *D. buzzatii* flies counted on the discs was significantly greater than that for *D. aldrichi*, the latter laid more eggs (difference not significant) and showed a significantly higher ratio of eggs per fly.

Table 13. Correlation coefficients (r) between total number of flies per yeast disc and number of eggs per yeast disc for each yeast, each species and overall
$*P < 0{\cdot}05$; $**P < 0{\cdot}01$

	d.f.	r		d.f.	r
Yeast			Yeast		
Y1	4	0·24	Y9	4	0·75
Y2	4	0·20	Y10	4	0·26
Y3	4	0·42			
Y4	4	0·55	Species		
Y5	4	0·91*	*D. buzzatii*	16	0·52*
Y6	4	−0·39	*D. aldrichi*	34	0·51**
Y7	4	−0·05			
			Overall	53	0·40**

The correlation coefficients between total number of flies per yeast disc and number of eggs per yeast disc, for each yeast, each *Drosophila* species and overall (Table 13) were positive and significant for both species, overall and for one yeast (Y5). The high correlation for Y5 resulted from its being the most attractive to *D. aldrichi* and having highest oviposition by this species.

Discussion

The results have been presented in terms of the attractiveness of a variety of species of yeasts to *Drosophila* adults, measured by counting the number of flies located on each yeast at regular intervals through one day. Two potential criticisms should be noted. Firstly, experimental flies had been maintained for up to about 40 generations on medium containing autoclaved *S. cerevisiae*. If the populations had adapted to metabolic products of this yeast species present in the medium, this might change the relative attractiveness of the wild yeast species, and the relevance of the results to natural populations could be open to doubt. However, the rank order of attractiveness of the yeast species was generally consistent over all experiments, which would argue against any effect of adaptation to *S. cerevisiae*. Secondly, the results may be technique specific and one problem has been alluded to already in relation to results of the crop assay in experiment 2. That is, the data do not indicate the length of time that individual flies remain on a particular yeast disc. Thus, a particular yeast may be scored as attractive, either because flies visiting it remain for long periods of time (feeding or oviposition behaviour) or because many more flies visit it but remain only briefly (exploratory behaviour). In the first case, the flies may feed or oviposit, and in the second do neither, so that the measured 'attractiveness' of any particular yeast species could be more apparent than real.

Nevertheless, while this caution should be noted in interpretation of the results, there were consistent effects which imply behavioural differences between sexes, between immature and mature flies, and between the two *Drosophila* species. Males and females were scored separately in experiments 1, 2 and 3. The effects of sex and

interactions involving sex were consistent and significant in experiments 2 and 3, but were not significant in experiment 1 (possibly due to differences in technique or smaller number of replicates, or both). More females than males were attracted to the yeasts, but it is the sex difference apparent in the significant sex × yeasts and sex × times interactions that is most interesting. In both experiments, although there were significant differences among yeasts and among times for females, none were significant for males. Although this may indicate that males showed no differential attraction to the different yeasts, and no changes over times, it is more likely a function of the smaller numbers of males attracted to the yeast discs, and a consequent lack of power of the statistical test to discriminate among yeasts or among times (see Tables 5 and 8). This implies that males were less attracted. Flies in experiment 2 were 1–9 days old, and although virgin, females may show some increased attraction due to 'oviposition' as well as feeding response. In experiment 3, the flies were 1–3 days old, so any sex difference should be only in feeding response. King and Wilson (1955) estimated the daily intake of mature ovipositing *D. melanogaster* females as 1·12 mg of yeast, equivalent to 30 average sized meals, and that 40% of the phosphorus intake in the yeast was used in egg production. Mature males would have a lower food requirement, firstly because they are not producing eggs and secondly because of smaller body size. Body weights of flies used in the experiments were not measured, but subsequently a sample was taken from the cages used for experiment 3 and average body weights of 3-day-old virgin progeny (raised under the same conditions as those in experiment 3) were for *D. buzzatii* males and females 1·530 and 1·854 mg, respectively, and for *D. aldrichi* males and females 1·794 and 2·074 mg, respectively. If food requirements were directly proportional to body weight, the average number of *D. buzzatii* males counted on the yeast discs should be 82·5% of the number of females, and for *D. aldrichi* it should be 86·5%. As the observed percentages were 28·1 and 33·9 for *D. buzzatii* in experiments 2 and 3, and 55·5 for *D. aldrichi* in experiment 3, it is clear that the differential attraction of the two sexes involves more than proportional differences in food requirements. The reason for the sex difference remains unknown, but may be a function of the experimental techniques or the laboratory environment, particularly as field experiments (Barker *et al.* 1981) have shown no differences in the attraction of the two sexes to the yeasts used here in experiments 1 and 2.

In experiments 3, 4 and 5, where both *D. buzzatii* and *D. aldrichi* were tested, the former species showed significantly higher attraction to the yeasts. As the body weights of *D. buzzatii* were less than those of *D. aldrichi*, this difference also cannot be ascribed to differences in food requirements. While *D. aldrichi* may be less active or spend less time on the discs than *D. buzzatii*, the difference may be related to differences in yeast-species utilization in the natural population. Extensive and regular collections over 4 years have been made at 'Yarrawonga', from where both the *Drosophila* and yeast strains in these experiments derive, and there are clear differences in the population dynamics of *D. buzzatii* and *D. aldrichi*.

Further, although the distribution of *D. buzzatii* in Australia apparently is co-extensive with the *Opuntia* distribution, *D. aldrichi* is more restricted, and is concentrated in the northern part of the *Opuntia* distribution. The 'Yarrawonga' population of *D. aldrichi* is an outlying and presumably marginal one, being separated by 900 km from the main distribution area (Mulley and Barker 1977). At 'Yarrawonga', *D. buzzatii* shows two peaks in population abundance (November and

March–April) and has been collected in all months of the year, although in low numbers in the winter months and in mid-summer (December–January). In contrast, *D. aldrichi* shows just one peak in abundance (March–April) and although it must maintain a continuous population, very few are collected at other times. As noted in Materials and Methods, the nine species of yeasts used in experiments 3, 4 and 5 were included in the 10 most commonly isolated from collections over a 15-month period, but there was significant seasonal variation in the yeast-species distribution over this period (Barker, Phaff and Miranda, unpublished data). Thus, while *Rh. minuta* var. *minuta* was the fifth most common in the 376 isolates from 'Yarrawonga' with 23 isolates, 21 of these were found in April, i.e. at the time of the annual breeding peak of *D. aldrichi*. As this species was most attractive for mature females of *D. aldrichi* in experiment 5, but least attractive for young flies in experiments 3 and 4, data on the actual yeast utilization in natural populations are required.

There were significant differences in the numbers of flies attracted to each yeast in all but experiment 5. Experiments 1 and 2 (five yeast strains tested, *D. buzzatii* only) were consistent in that *P. cactophila* was most attractive and *C. sonorensis* [Y1(*b*)] least so, and were consistent with experiments 3 and 4 (nine yeast strains, both *D. buzzatii* and *D. aldrichi*) where *P. cactophila* and *L. opuntiae* were most attractive, and *C. sonorensis* together with *C. mucilagina* and *Rh. minuta* var. *minuta*, least attractive. Clearly, attractiveness of the yeasts was not related to their abundance in the natural environment.

Although there was no evidence in experiments 3–5 for differential attractiveness of the yeasts for the two species of *Drosophila* (non-significant species × yeasts interaction), there were consistent trends and differences in experiments 3 and 4 (1–3-day-old flies) as compared with experiment 5 (older mated females) that are suggestive and encourage further study. In experiments 3 and 4 where, as noted above, *P. cactophila* and *L. opuntiae* were highly attractive for both species, *P. opuntiae* var. *opuntiae* was highly attractive for *D. buzzatii* but only of intermediate attractiveness for *D. aldrichi*, while *Cr. cereanus* variety was highly attractive for *D. aldrichi* but of low to intermediate attractiveness for *D. buzzatii*. *Rh. minuta* var. *minuta*, *C. sonorensis* and *C. mucilagina* were of low attractiveness for both species. For *D. buzzatii* in experiment 5, these three species remained poorly attractive, and while *L. opuntiae* was still most attractive and carried the highest number of eggs per fly, *P. opuntiae* var. *opuntiae* was of only intermediate attractiveness, and *P. cactophila* was of low attractiveness.

In contrast, changes between the two sets of experiments were much more marked for *D. aldrichi*. The three yeast species that were most attractive for young flies were of low to intermediate attractiveness for mature females (although *P. cactophila* had the highest number of eggs per fly), while *C. sonorensis* and *Rh. minuta* var. *minuta* were the most attractive yeasts for mature females. Consequently, there is an indication of species separation in the utilization of these yeasts by mature females: *L. opuntiae* is highly attractive for *D. buzzatii*, but not for *D. aldrichi*, with the reverse pattern for *C. sonorensis* and *Rh. minuta* var. *minuta*. It may be particularly significant that *Rh. minuta* var. *minuta* was the most attractive yeast for mature *D. aldrichi* and had the most eggs laid on it (although not, as noted above, the highest number of eggs per fly) because, as noted previously, most isolates of this yeast were found at the time of the annual breeding peak of *D. aldrichi*. In addition, the contrasts in attractiveness of some of the yeast species for young flies and for adult females

suggest possible larval–adult niche separation. However, clarification of this possibility will depend on studies of larval preferences and the nutritional adequacy of the yeasts for larval growth and development, as the yeasts most attractive for adults may not necessarily support larval growth, while larvae show preferences for those yeasts on which they grow best (Cooper 1960).

In every experiment, the numbers of flies counted on the yeast discs changed during the day, being lowest in the early times and then generally increasing (Tables 2, 5, 8, 10). For the young, or virgin, or both types of flies in experiments 1–4, where presumably only feeding responses are involved, this suggests a delayed recognition of the yeasts as food sources. Results of experiments 3 and 4 are consistent with this suggestion in that in the former (flies prestored for 20 h on a non-yeast medium), the average numbers of flies on the yeast discs in period 1 (Table 8) are considerably higher than in the latter experiment (flies stored on dead yeast medium—Table 10), particularly for *D. buzzatii*. The increase in attraction may be caused by some kind of aggregation or cumulative conditioning effect with more and more flies being attracted to discs already utilized, confounded with direct feeding responses, or it may be a function of yeast growth during the day.

However, the attraction of the flies to the different yeast species changed over time, adding a further dimension of complexity to the observed responses. As the attractiveness of some yeasts remained stable over time or actually decreased, there cannot have been a generalized aggregation or cumulative conditioning effect, although such effects may have occurred for other yeasts. In experiments 1 and 2, only the yeast which was on average most attractive (*P. cactophila*) showed increased attractiveness over time. With the wider choice of yeast species in experiments 3 and 4, both the least and the most attractive species within each experiment tended to be stable over time, although the same species were not the most attractive in both experiments. The differences between these experiments probably relate to differences in pre-experimental conditions (storage without or with yeast in the medium), with flies in the former case (experiment 3) showing higher feeding responses at the beginning of the experimental observations and consequently less initial discrimination among yeast species. Thus, two species (*L. opuntiae* and *P. opuntiae* var. *opuntiae*) which increased in attractiveness over time in experiment 3, were highly attractive throughout in experiment 4.

Apart from the effects of time and yeasts × times, there were differences between the two *Drosophila* species (significant species × times interaction in experiments 3–5) and differences between young and mature flies. In experiments 3 and 4 (1–3-day-old flies), effects of times (or periods) were significant for *D. buzzatii* (generally increasing from period 1), but not for *D. aldrichi* (Tables 8 and 10). For mature females in experiment 5, however, the average number of *D. buzzatii* per disc increased in the order of period 3, 4, 1, 2, while the number of *D. aldrichi* increased significantly through periods 1–4. The differential changes in pattern for the two species between young and mature flies serve to emphasize the complexity of the behavioural responses involved, rather than providing the basis for any explanation.

On the assumption that there would be differences among yeasts in attractiveness for mature females, it was expected that relating the number of eggs per yeast disc to the total number of flies per yeast disc would help to differentiate feeding and oviposition responses. As there were no significant differences among yeasts for either variable, one has only the significant but not surprising, correlations between

them for each species and overall. However, as these correlations were only about 0·5, it is clear that other behavioural responses, as well as oviposition choice, have had a major effect on the observed numbers of flies on each yeast disc. In particular, responses of mature females also will involve choice of yeasts for feeding, and may involve possible aggregation or cumulative conditioning effects for feeding and aggregation behaviour for oviposition [as demonstrated by del Solar and Palomino (1966) for *D. melanogaster*, and del Solar (1968) for *D. pseudoobscura*]. It is now known that *D. buzzatii* females oviposit mainly in the late afternoon and early evening, so that if specific responses to different yeast species for oviposition do exist, they may be separated from other responses by providing mature well-fed females with a choice of yeast species for only a short period of time during this peak oviposition period.

Nevertheless, these experiments have shown differential attractiveness of yeast species isolated from the natural breeding and feeding habitat of *D. buzzatii* and *D. aldrichi* for young adult flies of these species, and have suggested separation between these species in the utilization of the yeasts by mature females. However, further studies of oviposition preferences, of the attractiveness of the yeast species for larvae, and of the ability of each yeast species to support larval growth and development, will be necessary to determine whether the yeast species are a significant component of environmental heterogeneity affecting selection in natural populations of *D. buzzatii* and *D. aldrichi*.

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