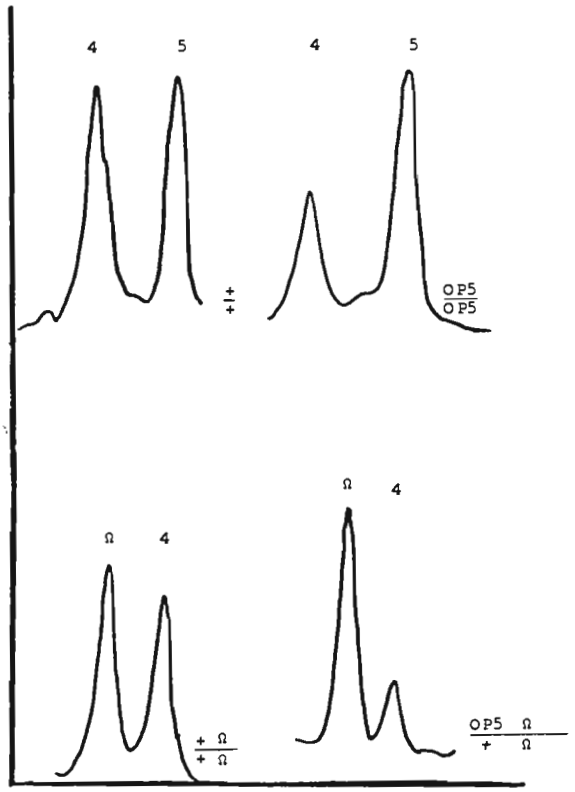

TABLE 1. Schema for the production of L_3CP5^{op} - Omega recombinants with results of the cross.

$$\frac{5^{op}}{5^{op}} \times \frac{omega}{omega} \text{ ------> } \frac{5^{op} \quad +}{+ \quad omega} \times \frac{+ \quad omega}{+ \quad omega}$$

		phenotype	#
Parentals -	$\frac{5^{op} \; +}{+ \; omega}$ ------>	5^{op}	20
	$\frac{+ \; omega}{+ \; omega}$ ------>	omega	19
Crossovers-	$\frac{5^{op} \; omega}{+ \quad omega}$ ------>	5^{op} omega	6
	$\frac{+ \quad +}{+ \; omega}$ ------>	+	1

Figure 2. Densitometer tracings as described in text.

While the numbers of recombinants are small they are consistent with an estimate of at least 15 map units between the omega and 5^{op} genes (Table 1). The gene for L_3CP-5 maps also maps at least 15 map units away from the Omega gene (Chihara & Kimbrell, in manuscript). The data are consistent with the L_3CP5^{op} locus being very close to the L_3CP5 gene.

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References: Fristrom, Hall & Watt 1978, Biochem 19:3917; Chihara, Silvert & Fristrom 1982, Dev.Biol. 89:379.

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Reproductive biology of **Drosophila buzzatii.**

Drosophila buzzatii and other cactophilic Drosophila provide a valuable model system for studies in population and evolutionary genetics (Barker & Starmer 1982), because they are specific to the cactus niche, feeding and breeding in rotting arms, fruits and cladodes of various cactus species. Studies of **D.buzzatii** in Australia have concentrated on allozyme polymorphism and factors affecting genetic variation at the allozyme loci. As a basis for study of the fitness of genotypes at allozyme loci, three preliminary experiments were done to provide data on the reproductive biology of the species.

In the first experiment, time to copulation, copulation duration, time from copulation to first egg and female fecundity in the 6.5 days after copulation were recorded. The flies used derived from 100 females collected at Breeza, N.S.W., and were generation 3 of mass culture in the laboratory. The medium used for culture and in all experiments reported here was autoclaved cactus-yeast-sucrose-agar. Using previously collected and stored (at 25°C) virgin flies, 82 pairs were set up (without etherization) over a 2.5 hr period (1400-1630 hr) at 20°C in empty 7.5 x 2.5 cm vials. All females were 30-48 hr old at initiation of these pair meetings, while males in the first 48 were 9 days old and in the last 34, 5 days old. Thirty-four of the 82 pairs had copulated by 1705 hr, when observation ceased. Because of the longer average observation period for 9-day old males, male ages were compared for proportion copulating in 30 min or in 60 min. Neither difference was significant, and the overall proportions were 0.207 and 0.244, respectively. Copulation duration ranged from 44 to 236 seconds. Again age of males had no significant effect and the overall average was 138.2±47.2 seconds.

Table 1. Egg production data for **D.buzzatii** females after an observed first copula.

Time from mean copula (days)	Proportions of females: Laying in this period	Laying for first time	Cumulative first laying	Mean no. eggs/ female	
0.08	0.03	0.03	0.03	0.62	6.88
0.58	0.41	0.38	0.41	6.26	
1.08	0.76	0.38	0.79	12.74	22.95
1.58	0.47	0.00	0.79	10.21	
2.08	0.76	0.12	0.91	37.15	47.15
2.58	0.50	0.03	0.94	10.00	
3.58	0.94	0.03	0.97	51.38	
4.58	0.91	0.00	0.97	50.97	
5.58	0.94	0.03	1.00	62.59	
6.58	0.91	0.00	1.00	48.32	

At the end of the observation period, small plastic spoons with medium and live **Saccharomyces cerevisiae** were added to each vial of the pairs that had copulated, and the pairs kept subsequently at 25°C. At 2000 hr, the flies were transferred to clean vials with fresh spoons. This was repeated at 12 hr intervals for 2.5 days, and then at 24 hr intervals for a further 4 days. The numbers of eggs on each spoon were counted after removal from the vials, and the spoons kept in plastic boxes at 25°C for 48 hr, when the numbers of unhatched eggs were counted.

With 1600 hr as the mean time of copulation, and taking 1800 hr and 0600 hr as mean egg laying times, egg production results (Table 1) are given for each period from the mean time of copulation. The average time from copulation to first egg (again male ages not significantly different) was 1.23±1.05 days. Delayed initiation of egg laying (up to 5.58 days) may have been due to an unsuccessful first copula, but with a later re-mating. The average total number of eggs per female for the 6.5 day period was 289.3±128.7, with a range of 23-449. Hatchability did not differ among egg collection periods, and equalled 99.6% of all eggs laid.

Markow (1982) has described sexual dimorphism for age at reproductive maturity in **D.mojavensis** and other cactophilic Drosophila species, with females reaching maturity some days earlier than males. As no data were available for **D.buzzatii**, the second experiment was set up to estimate age at reproductive maturity for each sex, indirectly as age at first egg-laying, by pairing young females with older males and vice versa. The flies used were again from the Breeza population at generation 3 of laboratory culture. Older flies, 5 days old when pair matings were set up, had been collected as virgins (< 22 hr old) under ether anaesthesia and kept at 25°C. Young flies, emerged during a 12 hr period (2000 hr - 0800 hr), were transferred without etherization to fresh media vials. From 1100-1200 on the same day, single pair matings were set up without etherization, in vials with egg-laying spoons containing cactus-yeast-sucrose-agar

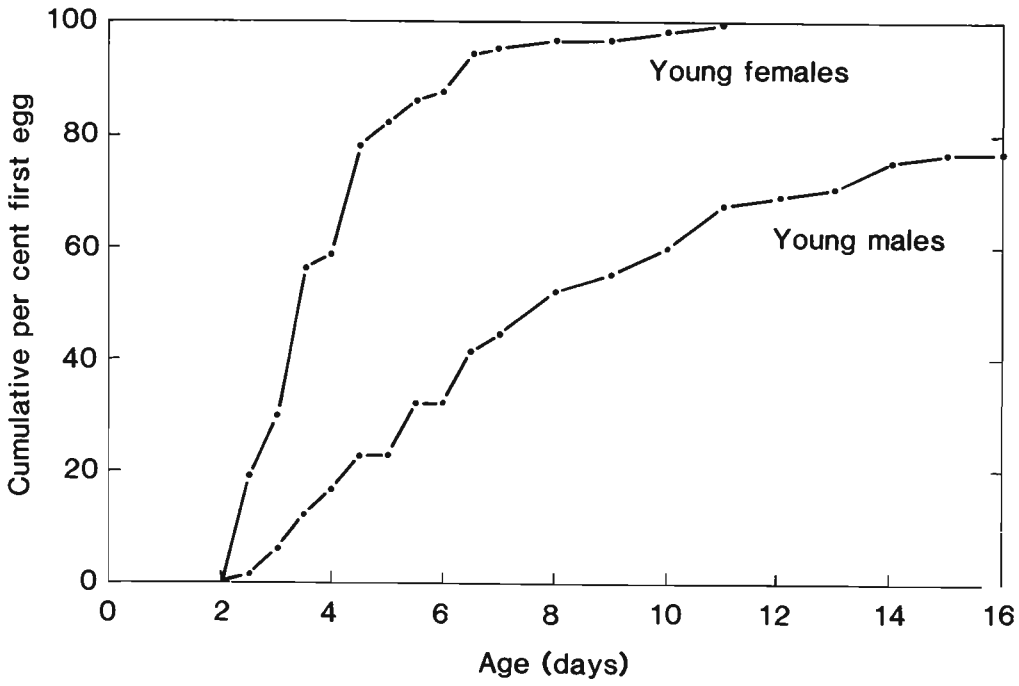

Figure 1. Cumulative percentage of first egg laying for young females paired with older males, and for young males paired with older females.

medium and live **S.cerevisiae:** 78 5-day old ♂ x young ♀, and 68 young ♂ x 5-day old ♀. At 2000-2100 hr that day, then every 12 hr for 7 days and subsequently every 24 hr, the pairs were transferred to clean vials with fresh spoons. After each transfer, spoons were checked for eggs. Where eggs had been laid, the numbers were counted and spoons kept as above to check hatchability. Pairs were discarded as they completed 3-4 days egg laying with normal hatchability. A number of vials were not included in the data set because of death of one of the pair, or loss of a fly at spoon transfer.

For six females, none of the first eggs laid hatched. These were assumed to be virgin eggs and were not counted as first egg-laying. Four of these females subsequently laid normally hatching eggs but no more eggs were laid by the other two.

Results were obtained for 73 young females paired with older males, and all had commenced egg laying by 11 days of age (Figure 1). The average age at first egg was 4.14±1.67 days.

For the young males with older females, only 77% of the pairs (50 of 65 possible) had produced eggs by the time the males were 16 days old (Figure 1). The average age of these 50 males at the first egg of their paired females was 7.33±3.41 days. The remaining 15 pairs that had produced no eggs were separated, paired with new 7-day old virgins, and kept until eggs were produced or until death (with virgin replacement where necessary). Eggs were produced by the females paired with nine of the 15 (originally) young males, but only three of the 15 (originally) older females produced eggs when paired with fresh males (and two of these laid only one egg).

These results show that male **D.buzzatii** reach reproductive maturity at a later age than females, but some of the apparent delay for males must be ascribed to continued rejection of younger males by older females. With this test system of continuous pairing and no choice, familiarity breeds contempt. Allowing for the estimated mean time from copulation to first egg (1.23 days), the average age of females at insemination was 3 days, while the average age at which males first successfully mated was at least 6 days).

The third experiment estimated lifetime fecundity and mortality schedules of flies maintained on two yeast species, viz. **S.cerevisiae** and **Pichia cactophila** (one of the two most common yeast species isolated from cactus rots in Australia: Barker, East, Phaff & Miranda 1984). The flies tested were first generation progeny of some 100 wild caught females from Trinkey, N.S.W. Pair matings were set up in

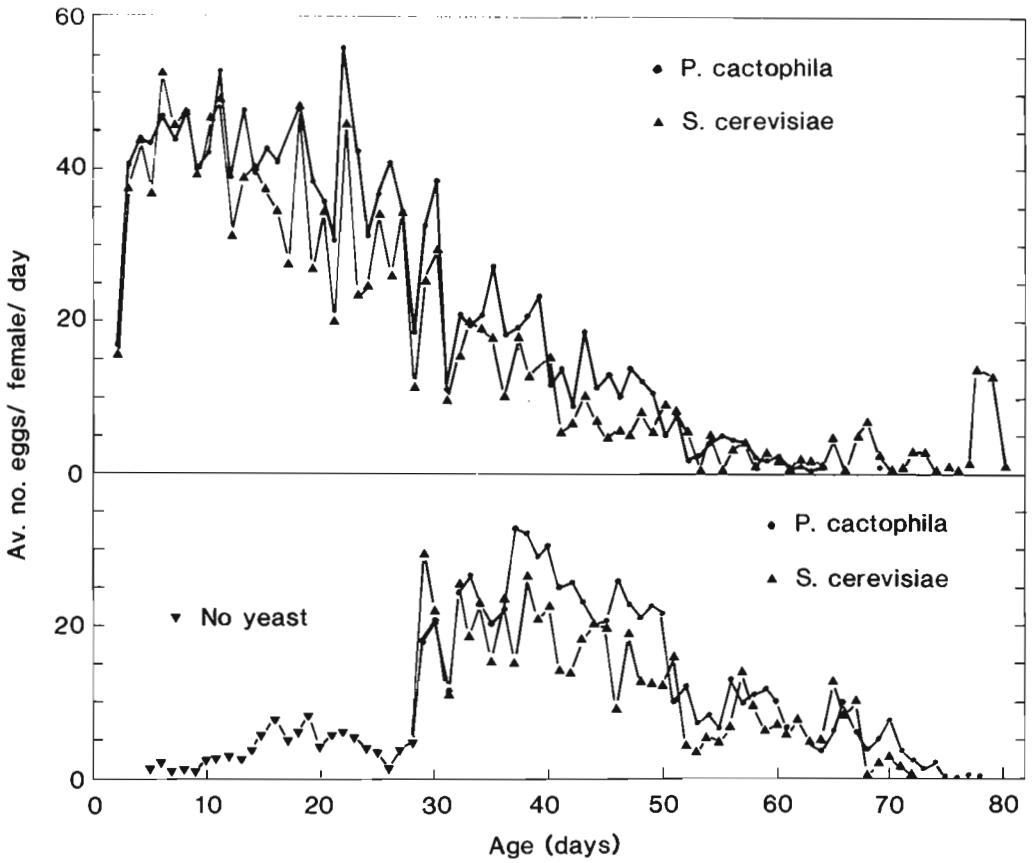

Figure 2. Fecundity schedules for females maintained on (1) **P.cactophila**, (2) **S.cerevisiae**, and (3) no yeast to 28 days of age, and then on **P.cactophila** or **S.cerevisiae.**

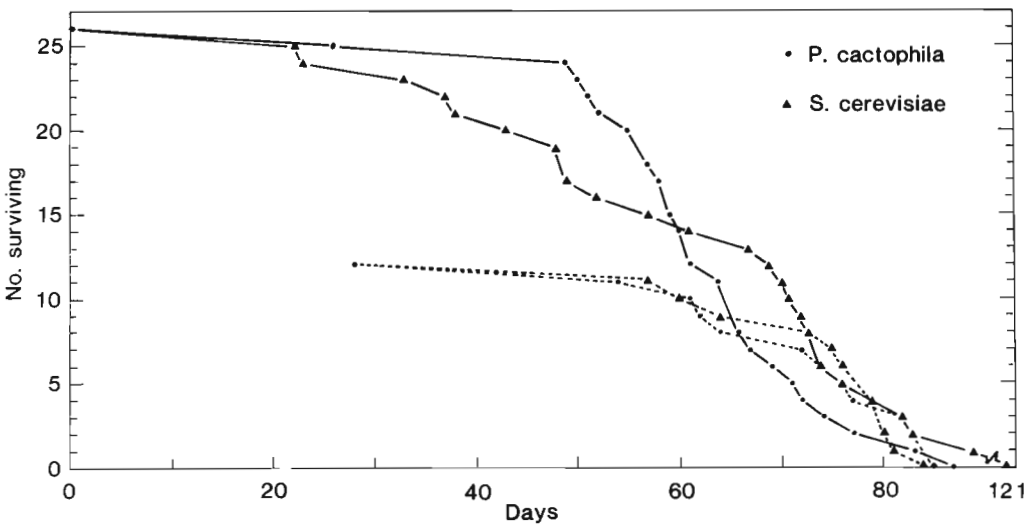

Figure 3. Numbers surviving for females maintained on (1) **P.cactophila**, (2) **S.cerevisiae**, and (3) no yeast to 28 days of age, and then on **P.cactophila** or **S.cerevisiae** (broken lines).

Table 2. Average number of eggs per initial female (standard deviation), cumulated to various ages, and to death of all females.

	Average eggs per female to ages:					
Treatment	9 days	16 days	23 days	30 days	37 days	TOTAL
P.cactophila	329 (133)	631 (254)	926 (368)	1157 (469)	1271 (530)	1455 (608)
S.cerevisiae	315 (164)	548 (306)	769 (449)	938 (531)	1035 (582)	1147 (649)

	28 days	35 days	42 days	
no yeast/ **P.cactophila**	100 (97)	252 (191)	434 (343)	712 (581)
no yeast/ **S.cerevisiae**		204 (176)	319 (286)	525 (538)

vials with egg-laying spoons as before, with the members of each pair deriving from different isofemale lines, but with approximately equal representation of the isofemale lines in each of three treatments: (1) **S.cerevisiae**; (2) **P.cactophila**; (3) no yeast. Twenty-six pairs were set up for each treatment, with males 3-7 days old and females 0-24 hr old. Spoons were changed daily and eggs counted. Average fecundity per day was calculated only for those pairs where both were still alive. Females on **P.cactophila** maintained early peak fecundity for longer and had higher total fecundity than those on **S.cerevisiae**, although the latter maintained egg production for longer (Figure 2 and Table 2).

The females maintained without yeast had very low fecundity (Figure 2), indicating that yeast feeding is almost essential for egg production. Egg numbers for the first 4 days are not shown in Figure 2, as the spoons for this treatment were inadvertently not changed until day 4. In this period, about half the females had laid, and the average number of eggs for all females was 25.2. Apparently some females had fed on yeast before being set in the pair matings. In the next 7 days, samples of spoons where eggs were laid were retained to check for yeast growth and hatchability of eggs. No yeast was detected and hatchability was normal. On day 28, the pairs of this treatment were separated in two groups,one subsequently maintained on **P.cactophila** and the other on **S.cerevisiae**. The numbers of eggs laid increased immediately, with average numbers/female/day higher than for females of the same age maintained throughout on live yeast. One or two females in each of the four groups laid no eggs, and the highest total egg numbers recorded were 2608 for **P.cactophila**, 2077 for **S.cerevisiae**, 1478 for no yeast/**P.cactophila** and 1716 for no yeast/**S.cerevisiae.**

The mortality schedules were determined for all females in each treatment. When a male died, he was not replaced, but the female was maintained until death. Survival curves for each treatment (Figure 3) show that survival on **P.cactophila** was very high to 50 days of age and then decreased rapidly, while on **S.cerevisiae**, the numbers surviving decreased more evenly from 20 days of age. For the no yeast treatment, one female died at seven days and one at 25 days, and the survival curves for the 12 females put onto each yeast at 28 days of age are very similar. The mean ages at death were 62.2±12.2 for **P.cactophila**, 61.9±22.0 for **S.cerevisiae**, 64.8±23.1 for no yeast/**P.cactophila** and 65.7±22.9 for no yeast/**S.cerevisiae.**

Although there are no differences in mean lifespan between females maintained on the two yeast species, the fitness of **D.buzzatii** is higher on the cactophilic yeast, at least in terms of higher initial average fecundity and survival. Comparison of these fitness components for flies maintained on other naturally occurring cactophilic yeasts would be of interest.

Work supported by a grant from the Australian Research Grants Scheme. We are grateful to Tom Starmer for assistance with copulation observation, Darren Schafer for egg counting and Jenny Druitt, Annette Royal and Chris Leger for patient preparation of thousands of egg-laying spoons.

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Bel, Y. and J. Ferré. University of Valencia, Spain. Regulation of eye-pigment metabolism in **Drosophila melanogaster:** effect of high doses of aromatic amino acids in the diet.

It has been reported that the "in vivo" hydroxylation of aromatic amino acids (phenylalanine, tyrosine and tryptophan) requires 5,6,7,8-tetrahydrobiopterin, a pteridine derivative. In **Drosophila melanogaster**, the synthesis of this cofactor shares some metabolic steps with the synthesis of the red eye-pigments (also pteridine derivates). For this reason we considered it interesting to see how the pteridine pathway responded to high doses of aromatic amino acids in the diet. Since tryptophan is a precursor of xanthommatin (brown pigment of **Drosophila** eyes), metabolites of this pathway were also analyzed.

Increasing doses of L-tyrosine, L-tryptophan and L-phenylalanine were given to the larvae till the moment of pupation. Pupae were transferred to new non-supplemented media and 9-day old adults analyzed for eye-pigment metabolites.

Viability was found diminished when phenylalanine was used (21 adults per 100 eggs with the highest load), whereas no change in viability was found in flies fed with similar doses of tyrosine and tryptophan. Tyrosine was also found not to affect developmental time. Tryptophan and phenylalanine delayed pupation and eclosion times. Phenylalanine caused longer delays and lower synchronization of the eclosion times.

Pteridines were measured in extracts of wild type flies raised in media supplemented with the different amino acids. Table 1 summarizes the results obtained with the highest loads (266.7 mg/ml of food). Regarding xanthommatin metabolites, xanthurenic acid increased when flies were fed with tryptophan and decreased in flies fed with phenylalanine. Xanthurenic acid 8-O-glucoside and kynurenic acid also appeared in chromatograms of flies fed with tryptophan (these two metabolites are not detected in chromatograms of the wild type raised in standard media). No change was found using tyrosine. Xanthommatin biosynthesis was already known to be enhanced when tryptophan was added to the medium, and inhibited when phenylalanine and tyrosine were used instead (Puckett & Petty 1980).

Table 1. Levels of pteridines in flies raised in media supplemented with different amino acids (266.7 mg/ml of food). "Drosopterins" estimation was carried out after selective extraction in acidified ethyl alcohol (Real et al. 1985). The other pteridines were estimated after thin-layer chromatography on cellulose. + = like in the non-supplemented control; 1- = diminished; 1+ = increased; values in parenthesis need further confirmation.

	Supplemented amino acid		
Pteridines	L-Tyrosine	L-Tryptophan	L-Phenylalanine
"Drosopterins"	(+)	1-	1-
Isoxanthopterin	+	1-	1-
H_2-Biopterin	1+	(1+)	1-
Biopterin	1+	+	1-
Pterin	1+	+	?
Sepiapterin	1+	+	1-
Acetyldihydrohomopterin	+	+	1-

The decrease of pteridine and xanthommatin biosynthesis in flies raised on phenylalanine media seems to be a consequence of a general toxic effect of this amino acid on the development of the insect. However it is worth noting that a blue fluorescent spot (probably a pteridine) appears overlapping with pterin in chromatograms of flies raised in phenylalanine media. The accumulation of this metabolite could be the result of a specific response of the pteridine pathway to phenylalanine loads. This possibility is currently being investigated in our laboratory.

References: Puckett, L. & K. Petty 1980, Biochem. Genet. 18:1221-1228; Real, M.D., J. Ferre & J.L. Mensua 1985, DIS 61 (this issue).