The effects of sterol mutants of the yeast *Saccharomyces cerevisiae* on the outcome of competition between *Drosophila melanogaster* and *D. simulans*

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Abstract

The effect of different yeast variants of the yeast *Saccharomyces cerevisiae* on the outcome of competition between *Drosophila simulans* and *D. melanogaster* was investigated. In all experiments differential birth rate was the effective mode of species competition. Addition of live yeast and especially mixtures of yeast strains improved the competition situation of a species, but could not prevent the extinction.

Introduction

In natural populations species are associated with each other and may compete for limited resources. The sibling species *Drosophila melanogaster* and *D. simulans* are sympatric and utilize yeasts for their essential food components (Sang, 1978; review). It has been claimed that genetic variability in *Drosophila* species is correlated with changes in the yeast environment (Powell, 1971; McDonald & Ayala, 1974; Bos *et al.*, 1976; Kircher, 1969). In view of the importance of yeasts in the nutrition of *Drosophila* and the differential attractiveness of different yeasts to sympatric species (Dobzhansky *et al.*, 1956), one might expect the relative competitive abilities of the species in laboratory populations to vary depending on the yeasts used in the culture (see for review of interspecific competition: Barker, in prep.). The abilities may sometimes be influenced by the composition of yeast species present in the culture as shown by El-Helw and Ali (1970) and El-Helw *et al.* (1972). Bos *et al.* (1976) found that species of *Drosophila* differ in their ability to utilize ergosterol-deficient mutant strains of the yeast *Saccharomyces cerevisiae*. In monoxenic cultures containing two of these mutant yeasts (*erg-2* and *erg-3*) the survival of *D. simulans* is

superior to that of *D. melanogaster*. One might expect from this that fixation of *D. melanogaster* as found in so many mixed populations of the two species (Moore, 1952; Tantawy & Solimar, 1967; Barker, in prep.), can at least be postponed in an environment which includes these sterol-mutant yeast strains. We report here the results of such competition experiments on mixtures of yeast strains with different sterol contents and discuss the effects of medium on interspecific competition.

Material and methods

Yeast strains

The yeast strains used in these experiments were a wildtype strain of *Saccharomyces cerevisiae* (A 18.4 A), two sterol mutants derived from this stock (*erg-2* and *erg-3*; formerly named *pol* mutants in Molzahn & Woods, 1972) and a commercial wild-type strain, originally bought as active dry yeast. The strains were grown in 2 litres Erlenmeyer flasks on a rotary shaker at 28 °C in 750 ml of yeast complete medium (Molzahn & Woods, 1972).

Drosophila stocks

Flies used for the experiments were taken from large laboratory massbred populations, maintained under a system of random mating since 1975. The *melanogaster* population derived from a two-way cross between Oregon and Novosibirsk strains, homozygous for the body colour mutation ebony. The *simulans* populations derived from an F2 of a cross between a Sheffield massbred strain and a white-eyed strain from Groningen. The population was homozygous for the eye colour mutant white eye. Culture temperature was always 25 °C.

Experimental procedures

Competition experiments

Competition was studied in four different experiments. Two series of Cage (A and B) and two series of Bottle experiments (C and D).

Cage experiments A. Eight types of cages were started november 1976, each with 1000 flies (250 ♀♀ and 250 ♂♂ from each of the species) and stopped after five months. The types of the cages are listed in Table 1.

Table 1. Types of cages used.

Cages	Yeasts			Standard* medium
	wildtype (A18.4A)	<i>erg-2</i>	<i>erg-3</i>	
A1	100%	–	–	–
2	–	100%	–	–
3	–	–	100%	–
4	–	–	–	100%
5	80%	20%	–	–
6	20%	80%	–	–
7	80%	–	20%	–
8	20%	–	80%	–

* 'standard medium' = 1000 ml water, 19 g agar, 32 g dead wildtype yeast, 54 g sucrose and 13 ml nipagin solution (10 g nipagin in 100 ml ethanol, 96%).

The environments are here identified by the percentage of mutant yeast they contain (e.g. 20% *erg-2*).

Live yeast was offered on the surface of an agar gel (2%) in food cups, different yeasts in different

cups. Every cup contained about 2 g of live yeast. Cups were replaced according to a scheme, which guaranteed that some fresh cups were provided every two days and that a cup stayed about 17 days in a cage. Sampling of eggs to estimate the proportion of the two species was done regularly (once a month) by inserting five fresh cups in each cage for one day. The emerging adults were collected and scored. Each type of cage was duplicated.

Cage experiment B. Experiment A was repeated between October 1977 and January 1978, because (1) in some cages contamination with fertile, wild type *melanogaster* was observed, although replicates with and without contamination reached the same final outcome – the fixation of *melanogaster*; (2) we wanted to test whether our sampling technique was reliable for estimating the proportion of the species present in the cages. In this experiment eggs were collected on slices of standard medium placed on corks in the cages. Slices with eggs were placed in standard food bottles and the number of flies hatching from these bottles was then determined. During the egg-collection period the number of flies visiting the slices was also counted, affording a second measure of the frequency of the species. Flies were counted 1 h, and 2 h, after insertion of the slices and the two counts were averaged.

Bottle experiment C. Experiment C was done in bottles, started in April 1978 and lasted five generations. Each bottle was supplemented with 5 g of live yeast on top of 30 ml of sucrose agar (5.4% sucrose; 2% agar). If a bottle was supplemented with two yeasts, they were added separately. The eight types of food medium were assayed (C1–8), with only one difference: the wildtype yeast was the commercial live yeast. A population on a medium consisted of four bottles with flies, started with 20 pairs of each of the two species per bottle. After two weeks the hatched flies were scored as *simulans* or *melanogaster* and then divided into four fresh bottles for about one day of egg-laying. An advantage of these experiments with non-overlapping generations was among others the fact that the population size and species frequencies could be determined every generation.

In addition to the eight types of media the effect of live yeast on top of standard medium was assayed.

Bottle experiment D. A large-scale bottle experiment was carried out during the winter of 1978–1979 for six generations. Two replicates of every experiment (D1–8) were used. For three generations all flies were counted and then used to start the next generation, but from generation four onwards every fresh bottle was started with 40 pairs of flies using the same frequencies as in the total population. In addition an environment containing 95% *erg-3* yeast was assayed.

Other procedures in the *D* experiment

Production of adults. The production of offspring by females was measured using vials (4 ♀♀, 3–5 days old) containing one of both species. Thirty replicates were made using standard medium (see experiment A) with both species together. On wild-type yeast (1 g live yeast on 9 ml of 2% agar) there were seven replicates for *D. melanogaster*, and twelve replicates for *D. simulans*. Egg-laying time was 24 h.

Development time. Measurements were made using twice-daily counts of hatching adult flies in bottles and the time halfway egg-laying period. Pure species cultures were given a short egg-laying time (8 h; 6–10 replicate bottles), mixed species cultures a long egg-laying time (20 h; 6 replicates) to measure egg-to-adult-development time in 'optimal' and competitive conditions.

Body weight. In the same experiment body weight was determined by weighing five groups of five males from each environment.

Results

Competition and yeast environments

The course of the proportion of *D. simulans* in competition with *D. melanogaster* in two cage

experiments (A and B) and two bottle experiments (C and D) is presented in Figure 1. Table 2 presents population sizes in experiments B and D after two months of competition. All sample periods are included. From these data five points are obvious:

- (1) In all the environments in experiments A, C and D the final result was extinction of *Drosophila simulans*.
- (2) In experiments B all the populations were fixed in *D. simulans* or had a high frequency, except on the 'standard' medium. These exceptional results of experiment B will be analyzed in part 2 of the results.
- (3) On *erg-2* and *erg-3* medium both species became extinct in all experiments except the B cages and one A replicate.
- (4) The decrease of the frequency of *D. simulans* was gradually on live wildtype yeast and the mixtures of live yeasts. On standard medium with killed yeast the decrease went much faster: e.g. the proportion of *simulans* in cages decreased from 50% to about 3% (9 angles) in 1 month. On live yeast the proportion was still at least 25% (30 angles) after one month of competition, which is about 1 or 2 generations (Fig. 1a). The use of live yeast apparently postponed the extinction of *simulans*.
The effect of addition of live yeast to the standard medium was measured in a separate experiment which was performed simultaneously with experiment D. The results are presented in Figure 2. These data confirmed the above conclusion that live yeast postponed the extinction of *D. simulans*.
- (5) Since within experiments and even between experiments A, C and D there was a good correspondence between replicates on the same medium, we calculated the average proportions of *D. simulans* (angles), averaged over experiments A, C and D after one and two months of competition ($\bar{x} \pm \text{s.e.}$) (Table 3).

Table 2. Population size (average of 2–4 replicates) in experiments B (cages) and D (bottles), two months after the start of competition.

	Yeast environment							
	1 100% + yeast	2 100% <i>erg-2</i>	3 100% <i>erg-3</i>	4 standard	5 20% <i>erg-2</i>	6 80% <i>erg-2</i>	7 20% <i>erg-3</i>	8 80% <i>erg-3</i>
Exp. B	396	77	326	252	407	188	543	445
Exp. D	213	0	0	297	515	397	591	459

Fig. 1. Proportion of *D. simulans* in competition: (a) Cages, * cages contaminated with *D. melanogaster*; (b) Bottles.

Table 3. Average proportions of *D. simulans* after one and two months of competition (exps A, C and D).

Mo.	Standard	80% <i>erg-2</i>	100% +- yeast	20% <i>erg-2</i>	20% <i>erg-3</i>	80% <i>erg-3</i>
1	11.2 $\pm$ 3.0	27.8 $\pm$ 5.1	32.2 $\pm$ 11.8	32.5 $\pm$ 9.2	29.7 $\pm$ 5.7	35.7 $\pm$ 4.3
2	2.8 $\pm$ 1.7	10.8 $\pm$ 4.2	13.4 $\pm$ 5.1	22.8 $\pm$ 9.5	28.2 $\pm$ 4.9	33.4 $\pm$ 8.5

After one month the proportion of *simulans* on 'standard' medium is significantly lower (t-test; $P < 0.05$) than the other proportions.

After two months the proportion on 'standard' is still significantly lower ($P < 0.05$) than the other proportions except '80% *erg-2*'. Comparing all pairs of treatments a difference of 10 units is significant (Tuckey's t-test; $P < 0.05$).

These data illustrated again the postponement of *simulans* extinction on live yeast but they also suggested that after two months of competition *D. simulans* extinction was postponed especially in the 'mixed'-*erg-3* environments compared to the standard environment (see also Fig. 1). This is demonstrated also in Figure 3. In this figure the differences between the proportion (average of replicates) of *simulans* on mixed *erg-3* and 100% *erg-3* yeast environments and on wildtype yeast are given. A short experiment on 95% *erg-3* is included here. It can be seen that with increasing amounts of *erg-3* the proportion of *simulans* increased.

The exceptional experiment B

In experiment B all the populations became fixed

or had a high frequency for *D. simulans*, except the two replicates on standard medium (Fig. 1a). This is in contrast with the results in experiments A, C and D, where *D. melanogaster* became fixed.

In experiment A the frequency of a species was determined by counting the numbers of individuals hatching from egg samples taken from the cages. In the case of experiment B frequencies were estimated in the same way, but also directly by counting the number of flies present on the oviposition sites. These 'direct' data are presented in Table 4. The two methods of frequency determination turned out to be the same: the correlation between the two determinations was high and significant ($r = 0.92$; $P < 0.001$). So the contrasting results reflected the real situations in the cages.

From the counts of adults in the cages and the numbers of offspring in the egg-collection samples the productivity (flies/♀) could be estimated (Table 5).

In the 'standard medium' cages *simulans* became extinct, in all the other environments *melanogaster* disappeared. Table 5 shows that in twenty-two out of 32 comparisons a lower production was associated with extinction. In four comparisons we scored

Table 4. Proportion in angles of *D. simulans* flies, visiting the oviposition sites in the cage populations (experiment B). Two cages (1 and 2) of each yeast environment.

Sampling period (month)	Yeast environment															
	1		2		3		4		5		6		7		8	
	100% +-yeast		100% <i>erg-2</i>		100% <i>erg-3</i>		standard		20% <i>erg-2</i>		80% <i>erg-2</i>		20% <i>erg-3</i>		80% <i>erg-3</i>	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
0	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
1	20	21	90	90	72	89	27	28	24	27	58	51	35	23	48	44
2	53	77	90	90	87	90	24	18	73	59	51	86	70	63	57	60
3	70	90	90	90	90	90	0	0	90	75	73*	90	88	58	80	88

* contaminated with *D. melanogaster* wildtype flies

Table 5. Productivity (flies/♀) in *D. melanogaster* and *simulans* in experiment B after one and two months.

Sampling period (month)	Yeast environment															
	1		2		3		4		5		6		7		8	
	100% +-yeast		100% <i>erg-2</i>		100% <i>erg-3</i>		standard		20% <i>erg-2</i>		80% <i>erg-2</i>		20% <i>erg-3</i>		80% <i>erg-3</i>	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1. <i>mel.</i>	6.0	7.3	0	0	0	0	1.3	1.5	1.1	3.6	1.2	0.5	8.8	5.8	2.5	4.7
<i>sim.</i>	10.5	12.2	0	0	1.4	0.3	0.2	0.1	0.8	5.5	1.2	0.9	14.9	5.3	11.5	1.2
2. <i>mel.</i>	1.9	3.8	0	0	0	0	7.0	6.0	1.1	0.9	6.5	0	2.5	1.5	1.5	1.2
<i>sim.</i>	8.2	2.5	1.0	2.6	1.3	1.4	0.3	0.3	3.4	2.5	2.0*	5.2	1.7	3.0	5.9	1.2

* contaminated with *D. melanogaster* wildtype flies

no difference in productivity, in six higher production led to extinction.

So, in experiment B productivity of females can have played an important role in the outcome of competition.

Another inexplicable feature in experiment B is the survival of a population after more than one generation on *erg-2* medium. In our bottle experiments C and D, our cage experiment A and previous papers (Bos *et al.*, 1976) populations became extinct after one generation. This and the fact that we recently noticed (unpublished) that condition of yeast cultures can have an extreme influence on the survival of *Drosophila* larvae, suggests that during experiment B conditions or quality of yeasts differed from conditions during the other experiments. These unknown exceptional conditions offered the advantage for *simulans* to outlive *melanogaster*. The possibility that after a period covering about 20 generations the gene pool of our *Drosophila* stocks also may have changed, can not be excluded.

Fitness characters in experiment D

In experiment D productivity of females cultured outside and inside competitive situations on wildtype yeast and standard medium was measured (Table 6). Here again we found a positive relation between production of adults and survival in competition: in experiment D the species with the lowest productivity (*simulans*) decreased in all bottle populations.

Development time was analyzed during this experiment and the relevant data, including significance of differences within species are shown in Table 7. The development time of both species was longer in the mixed species, high-density conditions and longer on standard medium. In the competitive situation *D. simulans* developed slower than *melanogaster* on standard medium (t-test, $0.05 > P >$

Table 6. Production of adult flies ($\bar{x} \pm \text{s.e.}$) per four females in single (1)- and mixed (2)-species cultures (Experiment D).

	Wildtype yeast	Standard medium	
	(1)	(1)	(2)
<i>D. melanogaster</i>	59.7 ± 5.3	61.1 ± 2.8	64.8 ± 1.9
<i>D. simulans</i>	52.5 ± 2.8	42.2 ± 2.2	42.4 ± 3.7

Table 7. Development time (hours) of males under 'Pure species, low density' and 'Mixed species, high density' conditions: average of replicates $\pm$ s.e. (Between brackets, number of replicates and total number of males).

	Wildtype yeast	Standard medium	P
<i>D. melanogaster</i>			
Pure, low	208.8 ± 0.5 (5, 187)	226 ± 3.8 (5, 150)	***
Mixed, high	253.5 ± 0.9 (5, 472)	258.8 ± 2.7 (6, 723)	n.s.
P (t-test)	***	***	
<i>D. simulans</i>			
Pure, low	209.4 ± 2.3 (4, 116)	216.3 ± 1.5 (4, 81)	**
Mixed, high	254.0 ± 1.5 (5, 820)	269.1 ± 4.7 (6, 303)	*
P (t-test)	***	***	

Significance probabilities for t-test (within species):

*** $0.005 > P > 0.0005$; ** $0.01 > P > 0.005$; * $0.025 > P > 0.01$

0.025). Body weight of males was measured using flies from pure *D. simulans* and *D. melanogaster* bottles (Table 8). Body weight of *melanogaster* males was larger on standard medium as well as on wildtype yeast (significance probability for t-test < 0.0005).

So, in experiment D we found indications that the following fitness components make *D. melanogaster* more successful on 'standard' medium: higher productivity, shorter duration of development and a higher body weight than *simulans*.

Discussion

The outcome of interspecific competition in continuous populations with overlapping generations, or with discrete generations, is known to be affected by environmental factors. Barker (in prep.) has reviewed e.g. the effects of temperature, population

Table 8. Body weight (mg/5♂♂) of *Drosophila* during experiment D on wildtype yeast and standard medium ($\bar{x} \pm \text{s.e.}$).

	Wildtype yeast	Standard medium
<i>D. melanogaster</i>	4.56 ± 0.07	4.49 ± 0.08
<i>D. simulans</i>	3.78 ± 0.05	3.82 ± 0.02
Sign. prob. for t-test	< 0.0005	

density, species frequency and medium. He suggests that 'one might expect the relative competitive abilities of *Drosophila* species in laboratory populations to vary depending on the yeast species used in culture, and that such studies could have considerable significance in analysing the realized niches of sympatric *Drosophila* species in natural populations. Yet few studies have been done'.

In this respect we found in an earlier study (Bos *et al.*, 1976) that *Drosophila* species differ in their abilities to colonize different environmental niches, measured by their ability to maintain themselves for five successive generations on different ergosterol mutant yeasts. This was done in the absence of interspecific competition in a medium containing solely a given strain of yeast. We found *D. simulans* superior to *D. melanogaster* on *erg-2* and *erg-3* yeast mutants of *Saccharomyces cerevisiae*. These strains contain different biological mixtures of sterols since the mutant strains used are blocked in ergosterol synthesis (Barton *et al.*, 1974; Woods *et al.*, 1974). What was tested were the effects of different mixtures of sterols in a biological package of yeast cells on the outcome of competition. From Cooke and Sang (1970) it is known that *Drosophila* has a sterol utilization similar to that of other phytophagous insects. Sterols serve a bulk requirement for which the steric criteria for utilization are relatively broad and a micronutrient requirement with a narrow steric specificity. The last requirement is only satisfied by sterols closely related in structure to cholesterol.

On standard medium, which was a killed-yeast sucrose medium, a fast extinction of *simulans* occurs in all experiments (Fig. 1, Table 4). This medium is clearly the most unfavourable one for *simulans* in a competitive situation, which is also evident from the increased development time (Table 7). Figure 2 shows that with the addition of increasing amounts of live yeast to standard medium, the competitive ability of *D. simulans* improves. There is no clear, overall difference in the final outcome of competition between the populations on mixtures of yeast strains and populations on pure wildtype yeast: in experiment A, C and D *Drosophila simulans* became extinct on both types of medium, whereas in experiment B this species outcompeted *D. melanogaster* in both types of environment. However, the data suggest that the extinction of *simulans* is postponed in the 'mixed' *erg-3* environments

Fig. 2. Proportion of *D. simulans* in competition bottles with Standard medium enriched with different amounts of live yeast.

(Fig. 3). This or the fixation of *simulans* might be expected from more successful development of *D. simulans* on pure *erg-3* yeast. It was known that the amount and quality of the medium (Claringbold & Barker, 1961; Barker, 1963) and yeast species used (El-Helw & Ali, 1970) can significantly affect the outcome of competition between *D. melanogaster* and *D. simulans*. In our present paper we show, that even differences between yeast strains can in-

Fig. 3. Differences in proportion of *simulans* between environments with increased amounts of *erg-3* yeast and the simultaneous controls on wildtype yeast (in angles). Standard errors of average survival are in the order of 0.9-2.1: (1) 100% *erg-3*; (2) 95% *erg-3*; (3) 80% *erg-3*; (4) 20% *erg-3*.

fluence the competitive abilities of the two species.

It is possible to improve the competitive situation for *D. simulans* artificially by giving *D. simulans* a three-day prelay (Van Delden, 1970). Van Delden noticed an increased competitive ability only after about 77 generations of competition. So, selection for competitive ability is extremely slow, which means that the genetic component of this character is mainly non-additive (Barker, 1973; Futuyama, 1970). Therefore it is unlikely that in our experiments fixation of *D. melanogaster* or *D. simulans* within a few generations was accompanied by a selective change in the genetic components of competitive ability.

During experiments B (cages) and D (bottles) we measured productivity of females and found that the final extinction of a species in the majority of comparisons was correlated with the lowest production of adults in the competitive situation (Tables 5 and 6). In experiment D we found that at least on standard medium low productivity of *simulans* was accompanied by long development time (Table 7) and low body weight (Table 8). This combination of fitness characters made the exclusion understandable. The addition of live yeast could postpone the exclusion of a species (Fig. 2) and the degree of postponement was dependent on the composition of the yeast (Fig. 3).

The exceptional extinction of *D. melanogaster* in the live-yeast situations in experiment B seems to be caused by a temporary high productivity of the *simulans* females during this experiment (Table 5). This relatively higher productivity of *simulans* females can have had a genetic cause: the gene pool of the flies from our laboratory stock, starting the B experiment may have had features differing from the starting material of our experiments A, C and D, since several generations separated the experiments. In this case, however, it is difficult to understand that within the B experiment *D. simulans* had a relatively high productivity in the live-yeast situations and became fixed, while they had a low productivity on standard medium and became extinct. Since we know that the physiological condition of our live-yeast media can influence extremely the survival of *Drosophila* larvae (Bos & Boerema, 1982) we assume an unknown environmental cause of yeast variation.

In conclusion, we think that in all our experiments differential birth rate was the effective mode

of species competition. Addition of live yeast and especially mixtures of yeast strains can improve the competitive situation of a species, but can not prevent the extinction.

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