

# Interspecific competition in *Drosophila*

## I. Reversal of competitive superiority due to varying concentration of ethanol

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The experiments reported here were designed to investigate the outcome of interspecific competition between *Drosophila melanogaster* and *D. simulans* in environments varying in one factor only. The factor chosen was ethanol concentration in the medium; the strains used were *D. melanogaster* SM5 (Series I), *D. melanogaster w, b, el* (Series II) and *D. simulans v* (both Series).

The results of competition over a number of generations, the longest experiment lasting 100 days, showed clear differences in the relative competitive abilities of the two species between the different environments. With no ethanol, *D. simulans* won in all replicates; with 8% ethanol, *D. melanogaster* won in all replicates; at intermediate (4%) concentration, the winning species was not consistent. Experiments in Series II showed very similar results to their counterparts in Series I despite distinct genetic differences between the strains of *D. melanogaster* used.

These results tie in with published work on (i) the relative susceptibility of single-species cultures of *D. melanogaster* and *D. simulans* to high ethanol concentrations, and (ii) distributional patterns observed in some field populations exposed to alcohols in their environment.

KEY WORDS:—interspecific competition—*Drosophila*—ethanol.

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## INTRODUCTION

The question of why a particular species, rather than one of its congeners, is present in any natural environment is usually very difficult to answer. This is largely because the relative fitnesses of a pair of species will be affected by a number of environmental variables, in the same way that the fitnesses of alternative genetic types will often be determined by a number of selective agents (see, e.g., Jones, Leith & Rawlings, 1977). In fact, selective differentials between species are likely to be much stronger and more numerous than those between genetic variants within species.

Clearly, some environmental factors will play a much greater role than others in the determination of the relative fitnesses of closely-related species. If, for any pair or group of species, such factors can be identified, and their effects measured, an explanation of the species-distributions would be brought considerably closer.

The present study is concerned with the effect of ethanol on the outcome of competition between the sibling species *Drosophila melanogaster* and *D. simulans*. The relative proportions of these two species have been shown to vary, in semi-natural vineyard populations, apparently in response to ethanol concentration (McKenzie & Parsons, 1972; McKenzie, 1974). It has been shown (McKenzie & Parsons, 1972) that *D. melanogaster* survives better than *D. simulans* when each is exposed to ethanol in single-species culture. A similar result has been found by David & Bocquet (1976). However, in general the result of interspecific competition is not predictable from the behaviour of single-species cultures. Put in terms of the well known Lotka-Volterra model, a knowledge of  $r$ 's and  $K$ 's provides no information about the  $\alpha_{ij}$ 's, i.e., the competition coefficients. Since *D. melanogaster* and *D. simulans* may well be competing, the effect of ethanol on competitive outcome is of substantial interest. If *D. melanogaster* is favoured by ethanol in mixed as well as single culture, and its relative frequency in nature increases in space and time along ascending ethanol gradients, as found by McKenzie & Parsons (1972), then the case for a direct causal link between one specified environmental variable and relative species-distributions in the field is strong, even though the route it takes (via competitive or simple physiological effects) may remain uncertain.

The comparative evolutionary biology of *D. melanogaster* and *D. simulans*, including the information available on the effect of environmental ethanol, has been reviewed by Parsons (1975).

## MATERIALS AND METHODS

*Stocks*

Throughout the experiments, a single stock of *D. simulans* was used. This was both genetically marked and outbred. It was obtained by interbreeding four strains from different localities. The marker was the vermilion eye (*v*) gene. For the first series of experiments, an inbred (and balanced lethal) stock of *D. melanogaster*, SM5, was used. This was employed only until a suitably marked and outbred stock of *D. melanogaster* was available. The latter stock, which was used for Series II, was, like the *D. simulans* stock, made by interbreeding four different strains. It was marked with genes for white eye (*w*), black body (*b*) and elbow wing

(*el*). It will be referred to as *D. melanogaster w*. The alcohol dehydrogenase status of the three stocks was determined by electrophoresing samples of adult flies taken from the three base-populations. The *D. melanogaster* SM5 flies were all heterozygous  $Adh^F/Adh^S$  (a consequence of the balanced lethal system). The *w*, *b*, *el* stock of *D. melanogaster* was fixed for the  $Adh^F$  allele (fixation in this stock being almost inevitable since the alcohol dehydrogenase locus is extremely close to the *el* locus which was derived from an inbred line). The stock of *D. simulans* used was also monomorphic; it was fixed for an allele producing a cathodally-migrating allozyme (at pH 8.6).

### Containers

The population-cages used were made from clear plastic boxes of dimensions  $17.5 \times 11.5 \times 6.0$  cm. They had six 30 ml glass bottles screwed into their undersides, and these contained the medium. Each cage had two muslin-covered airholes of 2cm in diameter.

### Resources

All resources were based on a standard medium (described in Arthur, 1977). Different resources were distinguished only by differing concentrations of ethanol. The levels used were 0, 4 and 8%, by volume. Each bottle contained  $25 \pm$  ml of the appropriate resource, and was replaced every three weeks; thus two bottles were replaced per week.

### Experimental conditions

The experiments were carried out in a constant temperature room maintained at  $25 \pm 1^\circ\text{C}$ . The relative humidity was not controlled, but fluctuated around 40%. The light regime was 16 h light, 8 h dark.

### Experimental procedures

Flies were anaesthetized using  $\text{CO}_2$ . They were counted, both initially and throughout the experiment, while on a porous plate through which  $\text{CO}_2$  flowed. In the first series of experiments a sample of the adult population was taken for counting. In the second series population sizes were smaller, so the whole adult population was counted. Counts were made every two weeks, except in a few cases which will be apparent in the Figures.

### Experimental design

In Series I, the design was three treatments (0, 4, 8% ethanol)  $\times$  two starting-frequencies (0.2, 0.8 *D. melanogaster*)  $\times$  three replicates. In the second series, fewer cages could be set up due to a shortage of adult flies. Here, the design was two treatments (0, 8% ethanol)  $\times$  two starting-frequencies (0.2, 0.8)  $\times$  two replicates. So the total number of experimental population-cages was 26. The actual numbers of flies used to start each cage were 160 of the commoner species, 40 of the rarer. In all cases the initial sex-ratio was 1:1 in both species.

Single-species cages were also set up with the stocks used for Series I (*D. melanogaster* SM5 and *D. simulans* v). There were three replicates for each of the 0, 4 and 8% ethanol levels, i.e., 18 single-species cages in all.

### RESULTS

The results of the Series I experiments may be seen in Figs 1 to 3, those of Series II in Figs 4 and 5. A number of clear trends have emerged:

- (i) *D. simulans* won consistently on standard medium in Series I and II (10 populations in all).
- (ii) The outcome was reversed with 8% ethanol: here, *D. melanogaster* won in all cases (again a total of 10 populations).
- (iii) In the experiment utilizing 4% ethanol (Series I), the winning species was not always the same, but *D. simulans* was successful in more cages than *D.*

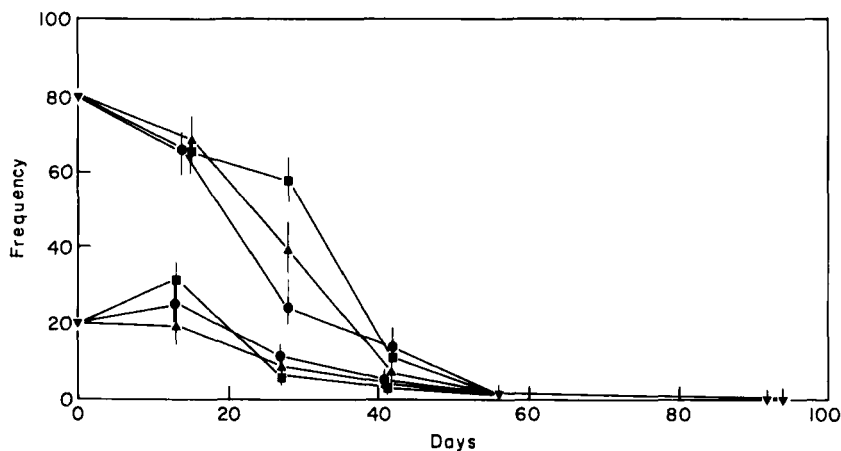


Figure 1. Results of experiment using standard medium: Series I. Frequency is expressed as % *D. melanogaster*. Bars indicate 95% confidence limits. ●, Replicate 'A'; ■, replicate 'B'; ▲, replicate 'C'; ▲ represents a point where frequencies in two or more cages coincide.

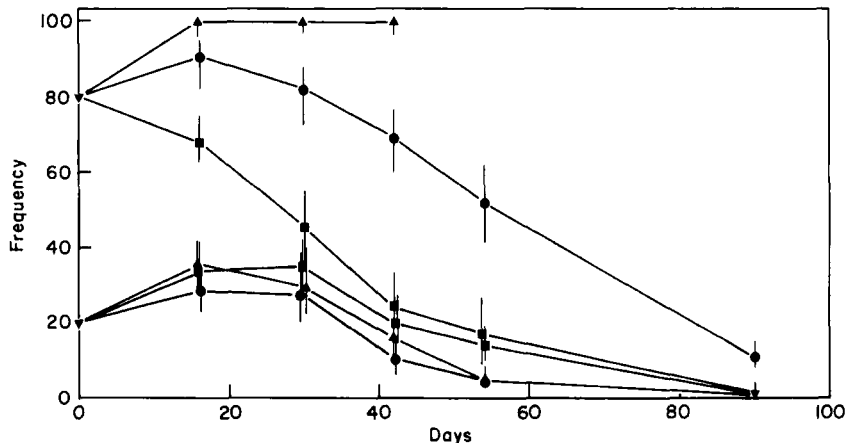


Figure 2. Results of experiment using medium with 4% ethanol: Series I. (See Fig. 1 legend for details.)

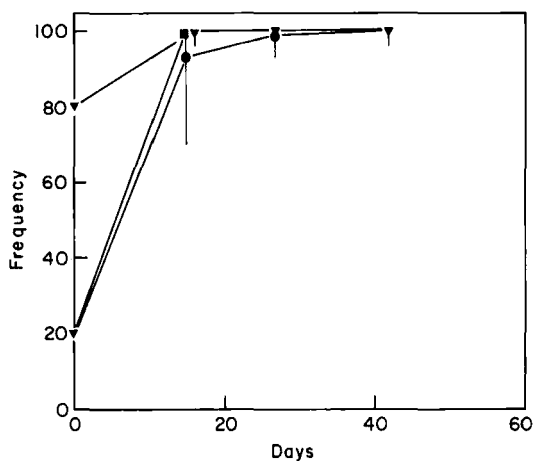


Figure 3. Results of experiment using medium with 8% ethanol: Series I. (See Fig. 1 legend for details.)

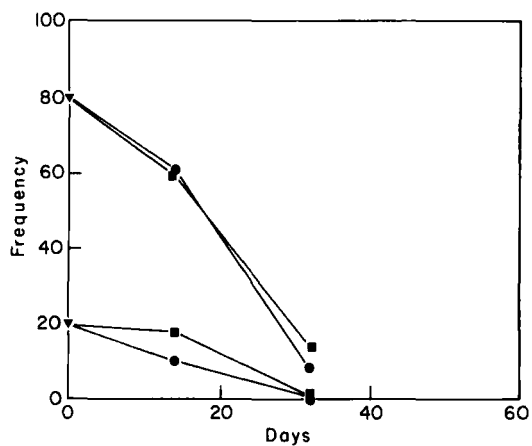


Figure 4. Results of experiment using standard medium: Series II. Frequency is expressed as % *D. melanogaster*. Confidence limits are not given as the total adult population was counted. ●, Replicate 'A'; ■, replicate 'B'; ▲, starting frequencies.

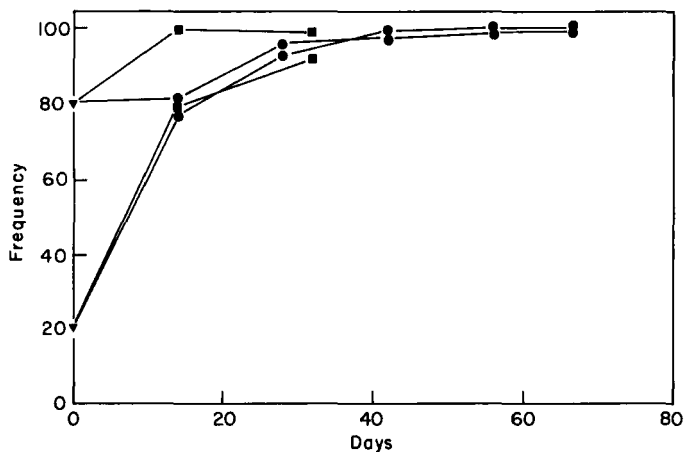


Figure 5. Results of experiment using 8% ethanol: Series II. (See Fig. 4 legend for details.)

*melanogaster* (five cages out of six). This sort of inconsistency is similar to that observed in experiments with *Tribolium* species in 'intermediate' environments (see, e.g., Park, 1962).

- (iv) In no case was there any sign of a stable equilibrium in species-frequency. This is in keeping with the generally accepted theory that the conditions for stable coexistence of two species on one resource are very restrictive. The result of the 4% ethanol experiment is consistent with an unstable equilibrium at about 80% *D. melanogaster*. However, other explanations are more likely (see below).

Single-species controls were established to ensure that the disappearance of a species in mixed culture—particularly of *D. simulans* in 8% ethanol—was in fact due to competition. Three replicate cultures of each species alone, in each treatment, were set up. Each cage was maintained up to the point when the corresponding mixed-species cages were terminated. The adult populations were then counted. In all cases, the single-species cages had around or in excess of 100 adult flies. Thus the extinctions in mixed culture can be attributed to competitive effects, rather than to a lack of physiological tolerance to the environments used.

Since *D. simulans* won in the majority of populations kept on 4% ethanol, it is worth testing whether the results of this experiment are in fact significantly different from those of the corresponding (Series I) experiment with standard medium. This can be done by comparing the cross-product-ratios (CPR's) for the two experiments. A CPR is a measure of the relative fitness of two species (or genotypes) and in the present study is:

$$\frac{N'_m \times N_s}{N'_s \times N_m}$$

where  $N$  = the number of adults in the  $i$ th generation,  
 $N'$  = the number of adults in the  $(i + 1)$ th generation,  
 and subscript  $m$  refers to *D. melanogaster*,  $s$  to *D. simulans*.

A CPR-value was calculated for each generation of each replicate.

For completeness, all comparisons between treatments (within series) and the two possible comparisons of equivalent treatments in different series were analyzed. Pairwise testing was performed using the Mann-Whitney U test. The results can be seen in Table 1. The U test is preferable, here, to a parametric equivalent since the CPR has a highly skewed distribution. (Its limits are 0 and  $\infty$ , with the two species being equally fit at CPR = 1.0.)

It can be seen from Table 1 that the 0 and 4% experiments in Series I were not significantly different in their CPR-distributions. Thus the possibility, mentioned earlier, of an unstable equilibrium at 0.8 *D. melanogaster* in the 4% experiment seems unlikely. A more probable explanation is that sampling smallish numbers simply resulted, in one cage, in a population of *D. melanogaster* of above-average competitive ability, or a below-average population of *D. simulans*. The latter alternative is more likely in that the number of *D. simulans* used to start the 'aberrant cage' was only 40, while for *D. melanogaster* the equivalent number was 160.

As regards the other statistical comparisons, they generally support conclusions that are immediately obvious from scanning the Figures. However, it should be noted that the Series I and II results for 8% ethanol differed significantly ( $P = 0.002$ ), while there was no significant difference between the

Table 1. Pairwise comparisons of CPR-distributions between different experimental regimes

Comparison between		$n_1$	$n_2$	U	z	P
1	2					
Series I: 0	Series I: 8	24	7	0.0	3.969	<0.001
Series I: 0	Series I: 4	24	26	245.0	1.301	n.s.
Series I: 4	Series I: 8	26	7	4.0	3.831	<0.001
Series II: 0	Series II: 8	8	12	3.0	—	<0.001
Series I: 0	Series II: 0	24	8	66.0	1.306	n.s.
Series I: 8	Series II: 8	7	12	7.0	—	0.002

$n_1$ , number of CPR's calculated for experiment in column 1;  $n_2$ , number of CPR's calculated for experiment in column 2. For any experiment,  $n = N - (x + y)$ ; where  $N$  is the total number of replicate-generations,  $x$  is the number of replicate-generations at the start of which one species is already extinct, and  $y$  is the number of between-count periods substantially longer than one generation (e.g., those at the end of Series I, no ethanol). U, the Mann-Whitney U statistic;  $z$  (the standard normal deviate), is used for determining  $P$  when  $n_1$  or  $n_2 > 20$  (see Siegel, 1956).

two series in the experiments on standard medium. Thus there seems to be an interaction between strain and alcohol level. If such interactions and/or non-additive interactions between different environmental factors are common, the determination of causality of reversals of competitive dominance in complex natural environments will be an even more difficult task than it already appears. However, the evidence for the interaction described here comes from comparing CPR-distributions, and most of the CPR's from the 8% ethanol experiments were measured in populations with extreme species-frequencies. This is problematic in that small changes in mortality could move a CPR from a small, finite value to infinity. Thus this particular result should be regarded only as suggestive.

A final point deserving mention is that the lack of a significant difference between the 0 and 4% experiments suggest that the response (of the differential in competitive abilities) to ethanol concentration is not linear. The most likely pattern of response, based on the results presented, would be a sigmoid one.

## DISCUSSION

### *Reversals of competitive dominance in Drosophila*

Reversal of competitive superiority by clearly specified environmental factors has not been commonly observed in *Drosophila*. Of the cases where reversal has been claimed, some studies do indeed show it, while others present a number of difficulties in drawing such a conclusion.

In the latter category, Ranganath & Krishnamurthy (1975) report an apparent 'reversal of dominance' between *D. nasuta* and *D. neonasuta*. Here, the supposed reversing agent is species-frequency, the system being said to exhibit inverse frequency-dependence, i.e., as the starting frequency of *D. neonasuta* increases, the competitive ability of the same species is said to increase. However, there are three main problems in accepting this interpretation. (1) In graphs of population sizes over time, the results of all four replicates of each treatment are added together and only the overall figures given. (The two treatments are starting frequencies of 1:1 and 1:4, *D. nasuta*: *D. neonasuta*). (2) Although in their table 1

the results for the different replicates are given separately, the results for different generations (within replicates) are averaged. Thus there is no information anywhere on how each replicate behaves in each generation (3) Regardless of this, the estimator of competitive ability used in their table 1 (mean number of males of each species) is partly a component of the variable actually used to create the two different treatments in the first place, so it is almost bound to be positively related to the starting frequency.

There are also several problems in accepting Ayala's (1966) 'reversal of dominance' in interspecific competition in three *Drosophila* species-pairs. *D. melanogaster* fared better at 19°C than at 25°C in competition with *D. serrata*, but the strains of *D. melanogaster* used were different. In the other species-pairs, strains in the two treatments were the same, but all experiments had only a single 'replicate'. Further, the claimed reversal of dominance, in three experiments, due to selection for increased competitive ability as the experiment progressed, is based on interpreting the point of greatest change in the direction of the species-frequency trajectory as an evolutionary change. This is an *a posteriori* division of the experimental time-period and is accompanied by no statistical analysis whatsoever.

Turning to studies whose evidence is less controversial, Moore (1952) has shown that temperature can reverse the dominant species in competition between *D. melanogaster* and *D. simulans*. At 25°C, *D. simulans* was consistently eliminated. Although at 15°C, *D. melanogaster* was not actually eliminated during the time covered by the experiment, its frequency became very low. This effect of temperature has also been shown by Tantawy & Soliman (1967). Moore (1952) has also shown that the age of the medium can have a strong influence on which species becomes dominant (old medium favouring *D. simulans*, new medium *D. melanogaster*).

Reversals of dominance in mixed populations of *D. melanogaster* and *D. simulans* in nature have also been described, though the environmental variables concerned are more complex, or unspecified. Hoenigsberg (1968) reported a very swift, and clear, temporal transition from a *D. melanogaster*: *D. simulans* ratio of 10:1 to 1:2. This was associated with a sudden drastic reduction in fruit-tree diversity in the sampling area caused by the construction of a road. An equally dramatic but less sudden reversal, again in favour of *D. simulans*, was observed by Tantawy, Mourad & Masry (1970). However, in this instance there was no marked environmental change associated with the alteration in species-frequencies. The authors' explanation that the reversal is "mainly due to genetic influences and environmental factors" is clearly rather uninformative.

#### *Problems in extrapolating from strain to species*

In field studies such as those described above, it has been difficult to identify the particular environmental agent(s) causing reversals of dominance in mixed populations. In laboratory studies, temperature (Moore, 1952), and now ethanol concentration, can clearly be seen to reverse competitive superiority between *D. melanogaster* and *D. simulans*. However, all such studies deal either with particular mutant strains or with samples of wild-type populations from particular localities. So the question arises as to what relevance the results have in general



for the species-pair concerned. This question is, of course, particularly pertinent when highly mutant strains, such as the SM5 and *w*, *b*, *el* stocks of *D. melanogaster* used here, constitute the experimental material. There are a number of ways of dealing with this question.

It is possible to repeat experiments on different strains/populations. However, the possible number of such 'replications' is limited and can still only cover a small subset of each species. In the present experiments, two different strains of *D. melanogaster* were consistent in their replacement by *D. simulans* on standard medium. It is likely, though, that many wild-type populations of *D. melanogaster* would supplant this particular *D. simulans v* strain on standard medium: indeed, preliminary experiments (unpublished) have strongly suggested this.

It may be argued that the differential effect of any environmental factor between two species will be in the same direction regardless of the strains used. In terms of *D. melanogaster*, *D. simulans* and ethanol concentration, the argument would be that as ethanol concentration increased, *D. melanogaster* would win more quickly, lose more slowly, or win rather than lose—depending on the strains used, i.e., 'reversals' are simply those cases where the differential is most clearly seen due to the choice of particular strains.

It is of course possible that if the various populations of a species differ genetically at a large number of loci, they may not respond similarly to particular environmental agents. In other words, firstly, the differential effect between two species of a particular agent need not be in the same direction in different populations; and secondly, different environmental factors might be important in determining the outcome of interspecific competition in different populations. If this is so, then the problem discussed in the introduction (identifying the *major* factors responsible for determining the relative success of congeneric species) may have to be approached anew in each different locality.

### *Concluding remarks*

The following conclusions can be drawn from the results of the experiments reported here:

- (i) Ethanol favours *D. melanogaster* in interspecific competition against *D. simulans*, at least in the experimental populations that were observed.
- (ii) A competitive effect such as this *could* cause the type of distributional pattern found in the field by McKenzie & Parsons (1972).
- (iii) Where, in the field, a reversal of dominance between *D. melanogaster* and *D. simulans* is found to be related to a more complex environmental change (for example, the change in the species-diversity of fruit trees in Hoenigsberg's (1968) study), alcohol may be one of the factors involved.

It will be apparent that the experimental system investigated here might usefully be extended to examine competition in 2-resource environments. In particular, the possible role of frequency-dependence (resulting from resource-heterogeneity) in maintaining a state of stable coexistence would be of interest. This topic forms the basis of a separate paper (Arthur, 1980) since the question of what balancing mechanism(s) maintain a (non-trivial) stable equilibrium in species-frequency is a very different one to the question of which species wins when there is no such equilibrium.

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