

Mating competition in artificial populations of *Drosophila melanogaster* polymorphic for *ebony*

II. A test for minority male mating advantage

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Summary

Mating competition experiments with *D. melanogaster* were performed in light and darkness under conditions mimicking those under which artificial populations show a stable polymorphism for the *ebony* gene (*e*). In both light and darkness a minority male mating advantage was found for *e/+* males in competition with wild-type males, but in light only when *ebony* was in the majority. Homozygous wild-type males showed minority advantage only in light and *ebony* males only in darkness.

1. Introduction

Minority male advantage has been suggested to be responsible for maintaining genetic polymorphisms in populations of *Drosophila* (Ayala & Campbell, 1974; Ehrman & Propper, 1978). The present study was undertaken to investigate whether minority male advantage could be responsible for the stabilization of the *ebony* (*e*) mutation in experimental populations of *D. melanogaster* (Teissier, 1947; Polivanov, 1964; Søndergaard & Sick, 1985; Kyriacou, 1985). The frequency of *e* attained in long-term experiments has been shown to be influenced by light (Jacobs, 1961; Søndergaard & Sick, 1985), and light-dependent differences in mating success between the *e/e*, *e/+* and *+/+* males have been observed both in mating experiments where the three genotypes occurred in frequencies corresponding to a Hardy-Weinberg distribution for an *e*-gene frequency of 0.5 (Søndergaard, 1985) and in standard mating competition experiments (Kyriacou, 1981).

2. Materials and Methods

The experimental design and the *D. melanogaster* stocks used in this investigation have been described previously (Søndergaard, 1985). The flies used in these experiments were extracted from an artificial population showing a stable polymorphism for *ebony* and they are therefore believed to have a randomized genetic background (except perhaps for genes closely linked to *ebony*). The *ebony* allele used has been kept for 15 years in our laboratory and is of unknown origin. Artificial mating populations were set up by

Table 1. Number of male flies of each genotype used in the mating experiments in order to mimic the Hardy-Weinberg distribution at different q_e values

| q_e | <i>e/e</i> | <i>e/+</i> ^a | <i>+/+</i> |
|-------|------------|-------------------------|------------|
| 0.01 | 0 | 6 | 294 |
| 0.1 | 3 | 54 | 243 |
| 0.2 | 12 | 96 | 192 |
| 0.3 | 27 | 126 | 147 |
| 0.5 | 75 | 150 | 75 |
| 0.7 | 147 | 126 | 27 |
| 0.8 | 192 | 96 | 12 |
| 0.9 | 243 | 54 | 3 |
| 0.99 | 294 | 6 | 0 |

^a A mixture of *e/+* and *+e* flies (Kyriacou, 1981).

mixing 300 virgin males (36 h old) with 100 virgin females (7 days old) in Bennett population cages (Frydenberg, 1962) thus mimicking the actual breeding size (Frydenberg, 1962), the presumed competition conditions (Søndergaard, 1985), and the physical environment under which such populations show a stable polymorphism for *ebony* (Søndergaard & Sick, 1985), but not age-dependent changes in male courtship (Kyriacou, 1985). Numbers of *e/e*, *e/+* and *+/+* males were mixed according to Hardy-Weinberg distributions for different *e*-gene frequencies (q_e), see Table 1. Care was taken to choose minority males randomly from culture vials (Markow, 1980). To avoid the disturbance of direct inspection and the possible negative effect on mating success by marking males (Bryant, Kence & Kimball, 1980; Kence, 1981) *ebony* females were used as the other mating partner. The

Table 2. Relative mating success \pm standard deviation (rms \pm S.D.) of e/e , $e/+$, and $+/+$ males at the indicated e -gene frequencies (q_e)

| q_e | No. of experiments | e/e | $e/+$ | $+/+$ |
|-----------------|--------------------|-------------------|-------------------|-------------------|
| Light | | | | |
| 0.01 | 14 | — | 0.73 ± 0.15^a | 0.30 ± 0.03 |
| 0.1 | 10 | 0.33 ± 0.15^a | 0.60 ± 0.06^a | 0.19 ± 0.03^a |
| 0.2 | 13 | 0.19 ± 0.03^a | 0.45 ± 0.04 | 0.20 ± 0.04^a |
| 0.3 | 10 | 0.15 ± 0.03 | 0.40 ± 0.05 | 0.20 ± 0.04^a |
| 0.5 | 10 | 0.11 ± 0.06 | 0.40 ± 0.08 | 0.26 ± 0.07 |
| 0.7 | 10 | 0.11 ± 0.02 | 0.43 ± 0.05 | 0.32 ± 0.05 |
| 0.8 | 10 | 0.14 ± 0.02 | 0.58 ± 0.06^a | 0.48 ± 0.06^a |
| 0.9 | 19 | 0.17 ± 0.03^a | 0.73 ± 0.08^a | 0.54 ± 0.14^a |
| 0.99 | 12 | 0.27 ± 0.04^a | 1.35 ± 0.46^a | — |
| Darkness | | | | |
| 0.01 | 12 | — | 0.62 ± 0.11 | 0.30 ± 0.02^a |
| 0.1 | 10 | 0.88 ± 0.17^a | 0.66 ± 0.07^a | 0.17 ± 0.02^a |
| 0.2 | 11 | 0.48 ± 0.07^a | 0.47 ± 0.06 | 0.15 ± 0.05^a |
| 0.3 | 10 | 0.44 ± 0.07^a | 0.45 ± 0.05 | 0.13 ± 0.03^a |
| 0.5 | 10 | 0.30 ± 0.03 | 0.48 ± 0.06 | 0.06 ± 0.04 |
| 0.7 | 10 | 0.33 ± 0.04 | 0.33 ± 0.04^a | 0.16 ± 0.02^a |
| 0.8 | 10 | 0.31 ± 0.05 | 0.37 ± 0.06^a | 0.16 ± 0.03^a |
| 0.9 | 21 | 0.28 ± 0.04 | 0.50 ± 0.08 | 0.24 ± 0.12^a |
| 0.99 | 12 | 0.30 ± 0.03 | 0.58 ± 0.15 | — |

^a Significantly different from the corresponding value at $q_e = 0.5$. Comparisons are based on maximum likelihood estimates of mating probabilities and tested by χ^2 -tests. The expected rms by random mating is 0.33.

genotype of the successful male mating partner could then be inferred by the phenotype of the offspring of the individual females. Sterile copulations are thus not included in the results. Males and females were allowed to mate for 24 h in either darkness or light (1000 lx inside the boxes); after this interval 90–100% of the females were inseminated. Double inseminations would not be registered in this set-up, but previous experiments had shown only 1–5% of the females are double inseminated under conditions used in these experiments. For each q_e value, the experiments were replicated at least ten times. Results are expressed as the relative male mating success (rms), i.e. number of females inseminated by a given male genotype divided by the number of males of this genotype present.

3. Results

The results of the experiments are shown in Table 2 and interpreted in Fig. 1. The curves in Fig. 1 have not been extended beyond q_e values of 0.9 and 0.1 since at the extreme q_e values only two types of males are present (Table 1). At these frequencies the relative male mating success (rms) of the majority males is identical (e/e vs. $+/+$) in light and in darkness. The minority $e/+$ males show significantly higher rms', and highest when competing with e/e males in light.

The rms' of $e/+$ males at low q_e values are light independent, whereas at $q_e > 0.5$ rms' are significantly higher in light compared to darkness. In light

the rms' of $e/+$ males at $q_e = 0.1$ and 0.8 are significantly higher than in the interval $0.2 < q_e < 0.7$ (no significant differences); the rms at $q_e = 0.9$ is significantly higher than the rms at $q_e = 0.8$. In darkness the rms of $e/+$ males is significantly lower at $q_e = 0.7$ and 0.8 than at other q_e values; at $q_e = 0.1$ and 0.9 the rms values are significantly different from those at $q_e = 0.2$ and 0.8, respectively. The results thus show that when $e/+$ males are rare (see Table 1) their mating success is higher than when they are more common.

It should be noted that the flat U-shaped curves drawn in Fig. 1 for $+/+$ males in darkness and e/e males in light are very tentative. First of all, the rms' of e/e and $+/+$ males are not significantly different at identical q_e values, and for $+/+$ in darkness all the rms values are identical except at $q_e = 0.5$ (see Table 2). For e/e in light the rms' at both high and low q_e (0.1, 0.2 and 0.8, 0.9, respectively) are significantly different from the values at $q_e = 0.3, 0.5$ and 0.7. In light the rms of $+/+$ males is significantly lower when they are in majority (i.e. $q_e = 0.3, 0.2, 0.1$) than at $q_e = 0.5$. When they are rare ($q_e = 0.8$ and 0.9) they show a significant increase in rms in comparison to the value at $q_e = 0.5$. In darkness these males show identical values at all q_e except at 0.5 which is lower than the rest. Under these conditions e/e males show significantly higher mating success when they are rare (i.e. at $q_e = 0.3, 0.2$ and 0.1) than when they are more common (i.e. q_e higher than 0.5).

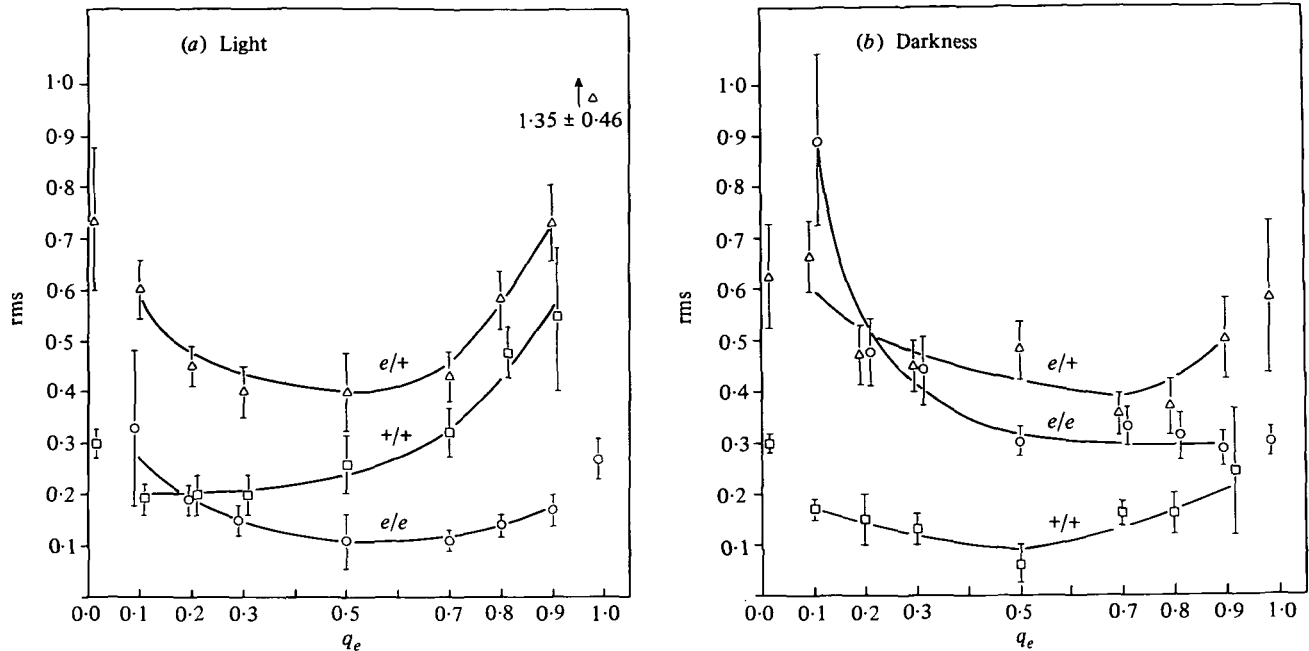


Fig. 1. Results of mating competition experiments mimicking those of artificial *D. melanogaster* populations. Males of different genotypes were mixed in accordance with Hardy-Weinberg distributions with different *e*-gene

frequencies (q_e). Three hundred males were mated to 100 *ebony* females for 24 h in (a) light or (b) darkness. o, *e/e* males; Δ , *e/+* males; \square , *+/+* males; rms, relative male mating success.

4. Discussion

The present mating competition results were obtained under conditions very close to those under which artificial populations of *D. melanogaster* have been shown to exhibit a stable but light-dependent polymorphism for the *ebony* gene (Søndergaard & Sick, 1985) (note however that all females are *e/e*). With respect to true frequency dependence in mating (Merrell, 1983) no conclusion can be drawn for *e/+* males since they constituted at most 50% of the male flies in these experiments, see Table 1. For *e/e* males evidence for frequency dependent mating success were found in light: the mating success at intermediate q_e values were significantly lower than at the two lowest frequencies. A clear minority male advantage was observed only for *+/+* males in light and *e/e* males in darkness. Wild-type males increase their mating success as their frequency decreases: lowest success at $q_e = 0.1$, highest at $q_e = 0.9$, with an intermediate rms at $q_e = 0.5$. Ebony males show unchanged mating success with q_e decreasing from 0.99 to 0.5, but at the three lowest q_e values (i.e. $q_e = 0.3, 0.2$ and 0.1) their success is significantly higher, and the rms at $q_e = 0.1$ is significantly higher than at $q_e = 0.3$, indicating that the rms is increasing in the interval $q_e = 0.3-0.1$. The light dependency of the minority male advantage in this case suggests that it is dependent upon a behavioural trait which is influenced by light. Since the mating performance of *e/e* males is light independent (Søndergaard, 1985) the minority male advantage could depend upon the behaviour of the wild-type males which, under the present conditions, are very

poor maters in darkness (Søndergaard, 1985 and Table 2), or alternatively, a light influenced change in female mating receptivity (Spiess, 1980).

Although some doubts have been thrown on the reality of the minority male advantage (Bryant *et al.* 1980; Merrell, 1983, see however Knoppin, 1984 and Petit, 1984) and difficulties in reproducing results have arisen (Partridge & Gardner, 1983; Spiess, 1982) there seems to be reason to believe that this phenomenon does exist (Anderson & Brown, 1984 and this investigation) although not universally (Ayala & Campbell, 1974; Pot, Van Delden & Krüyt, 1980; Markow, 1978; Markow *et al.* 1980), and that it could be of importance for the long-term retention of the *ebony* gene at low frequencies in experimental populations of *Drosophila*.

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