

Selection for oviposition preference in *Drosophila melanogaster*

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Summary

Populations of *Drosophila melanogaster* were subjected to selection for differing oviposition preference under allopatric and sympatric conditions. Flies were presented with the choice of a potato-based medium and a medium containing sugar and killed yeast on which to lay their eggs. Some gene flow was possible under sympatric conditions. In the allopatric lines selection was successful in rapidly generating an increased preference for sugar, and in the sympatric lines divergent oviposition preferences were generated in two cases out of four. A significant degree of reproductive isolation between one pair of allopatric lines was generated after eighteen months of selection.

1. Introduction

The neo-Darwinian view of speciation suggests that geographical isolation is necessary for speciation. Mayr (1963) argued that geographical isolation was essential for speciation, and his views were supported by those of Bigelow (1965) and Hansen (1966) who suggested that gene flow would prevent divergence within a population, and that therefore the only possible mode of speciation would be allopatric. The mechanics of allopatric speciation are discussed by Mayr (1947, 1963) and Dobzhansky (1970). Since then several investigators have been successful in producing partial reproductive isolation in populations that have been subjected to divergent selection under allopatric conditions (Hurd & Eisenberg, 1975; Soans, Pimentel & Soans, 1974), whereas others have produced divergence without reproductive isolation (Barker & Cummins, 1969).

A number of theoretical models of speciation, and of the genetic changes which accompany it, have been reviewed by Barton & Charlesworth (1984). All but one of the models they described involve geographical separation, either allopatric or parapatric, during the period of divergence. The exception is sympatric speciation. The possible features of a sympatric process of speciation were outlined by Maynard Smith (1966). A stable genetic polymorphism is possible if the habitat contains two niches, if density-dependent controls on population size operate separately in each

niche, and if the selective advantage of being in the right niche is sufficiently great. Then if individuals show habitat selection for one of the two niches, genetic differentiation could lead to the divergence of two reproductively isolated subpopulations. For example Guttman *et al.* (1981) described six races of the hymenopteran *Enchenopa binotata* defined by host plant range, which differed at a number of electrophoretic enzyme loci.

We report here the generation of genetically controlled differences in habitat choice by artificial selection. The experiments were designed to allow us to detect any reproductive isolation which might have developed in the process. Populations of *Drosophila melanogaster* were subjected to divergent selection for oviposition site preference under both allopatric and sympatric conditions. Jaenike (1982) has reviewed the experimental evidence for genetic control of this character, and has discussed the properties of the site which might be important. Consideration of such features as texture, odour, and available nutrients led us to choose the potato and sugar/yeast media described below.

In order to be able to monitor the extent of assortative mating in our populations, we backcrossed the allelic eye colour genes *brown* (*bw*) and *brown-4* (*bw⁴*) and also the gene *scarlet* (*st*) into our founder populations. In flies which are homozygous for *st*, the genotype *bw/bw* produces white eyes, the genotype *bw⁴/bw⁴* produces red eyes, and the eyes of the heterozygotes range from yellow to orange.

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2. Materials and Methods

(i) Founder stocks

The wild-type stock used was the IV stock, described by Charlesworth & Charlesworth (1985), and described by them as segregating at the loci for the enzymes alcohol dehydrogenase, phosphoglucomutase, and α -glycerophosphate dehydrogenase. It was kindly supplied by Dr Brian Charlesworth of the University of Chicago, USA. A stock homozygous for the genes bw^A and st was obtained from the Mid-America Stock Center, Bowling Green, Ohio, and the allele bw was available in Leicester.

The eye colour genes were crossed into the IV stock by a repeated backcross–intercross cycle. Orange-eyed flies with the genotype $bw/bw^A;st/st$ were crossed to the IV stock to produce an F_1 generation with genotypes $bw/+;st/+$ and $bw^A/+;st/+$. When these flies were intercrossed, one-eighth of the progeny had orange eyes, and these were then crossed to the IV stock to restart the cycle. This was repeated for nine complete cycles. The resulting founder population, designated Leicester 1, homozygous for st and segregating for bw and bw^A , was used in all subsequent selection experiments. Its genetic variability was checked using cellulose acetate electrophoresis (Bird & Semeonoff, 1982). It was found to be segregating at the enzyme loci examined by the Charlesworths (1985), with the frequency of the more common allele being approximately 0.75 in each case.

Throughout all the experiments described below, carbon dioxide was used when required to immobilize the flies.

(ii) Oviposition and culture media

Three different media were used (Bird, 1984). For the potato-based medium, each vial contained 4 g 'instant' mashed potato, reconstituted with 11 ml water containing 1% acetic acid. The sugar/yeast medium contained 10% sucrose and 2% dried yeast killed by boiling in suspension. The normal laboratory medium, used to raise the initial stocks and in periods of relaxed selection, contained 13% ground oats and 6.2% black treacle. All media contained a small amount of 'Nipagin' mould inhibitor.

(iii) Population cages

All selection experiments were carried out in cages made from clear plastic sandwich boxes, 115 × 175 × 60 mm deep. Eight holes in two rows of four were cut in the base of each box, and in these food vials were held in place by foam plastic collars. Two holes in the lid of each box were plugged with foam plastic plugs and allowed access to the cages. The populations were kept in these cages in continuous darkness at 26 °C. Preliminary experiments using the unselected Leicester 1 stock had shown that red-eyed

flies had an advantage over white-eyed flies in courtship, but that this advantage disappeared in the dark. This result is consistent with those reviewed by Tompkins (1984). The populations were maintained by replacing all the vials every three or four days. Dried yeast was added to the old vials as they were removed to accelerate the growth of the larvae. These vials were incubated at 26 °C for a further 8 to 12 days until the adults had emerged.

(iv) Selection in allopatric populations

Each selection line was founded with 150 females and 150 males from the Leicester 1 stock. Two lines were established for each eye colour, and for each eye colour one line was selected for preference for the potato medium, and the other for the sugar medium. This gives a total of four lines. These were: RS, red-eyed flies selected for sugar; WS, white-eyed flies selected for sugar; RP, red-eyed flies selected for potato; WP, white-eyed flies selected for potato. The lines were set up in March 1982 and remained isolated from each other throughout the period of the experiment, and are therefore described as 'allopatric selection lines'. From their initial starting size each line rapidly increased in numbers and maintained a constant population size of approximately 1000 flies.

Selection was performed as follows. Of the eight vials in a population cage, four contained the potato medium and four contained the sugar medium. The two media were arranged in a diagonal pattern to minimize any position effects. Populations subjected to selection in favour of oviposition on the potato medium and on the sugar medium are referred to as 'potato lines' and 'sugar lines' respectively. In the potato lines, when the old vials were removed from the cages, those containing potato medium were kept until the adult flies had emerged. These adults were then returned to the cage. The sugar lines were maintained in an analogous way.

(v) Selection in sympatric populations

The sympatric cages were first set up in June 1982, using 400 heterozygous (orange-eyed) flies, so that the progeny in the subsequent generation contained all three eye-colour genotypes. The cage 4P2 was re-established in August 1982 after the original cage had been contaminated with wild-type flies.

Selection was carried out as follows. The cages were set up as for the allopatric populations. After the old vials had been removed from the cages, all eight were kept until the adults had emerged. In the populations designated 4P1 and 4P2 we returned to the cages the $bw^A;st$ (red-eyed homozygous) flies emerging from eggs laid on the potato medium and the $bw;st$ (white-eyed homozygous) flies emerging from eggs laid on the sugar medium. Equal numbers of each sex of both selected genotypes were returned to the population,

and flies which emerged in vials containing the 'wrong' medium were discarded, as were all heterozygous flies. In the populations designated 4S1 and 4S2 the eye colour and medium combinations were reversed. Thus in each case divergent selection pressures were imposed within single populations. Because only flies homozygous for the eye-colour genes were preserved, the imposed selection was also in favour of positive assortative mating. Since most of the females had mated before being returned to the cage, this selection was only partial. Let us assume that a female lays all her eggs on the 'correct' medium. If this (necessarily homozygous) female has mated with a male with the opposite homozygous genotype, all her female progeny will be lost. If she has mated with a heterozygous male, approximately half her female progeny will be lost. If she has mated with a male of the same genotype as herself, all her progeny which reach adulthood will be preserved.

That this selection procedure makes possible a certain amount of gene flow can be seen as follows. Suppose a white-eyed female mates with a red-eyed male. Then if an orange-eyed male from the progeny of this mating mates with a red-eyed female, in the red-eyed female progeny from this mating one-quarter of their autosomal genes and one-half of their X-chromosomal genes will have been inherited from their white-eyed grandmothers. The process depends on the production of heterozygous males, in which of course there is no recombination. This means that the second chromosome, which carries the brown alleles, will be inherited intact and will not be involved in this gene flow. The rate of gene flow described above is the maximum possible.

(vi) *Measurement of oviposition site performance*

Preliminary experiments had shown that the number of pupae provided a valid indication of the number of eggs laid in a vial. Egg to adult survival was constant and high over all lines, between the two media, and between the beginning and end of the selection period. The total numbers of eggs varied from one generation to the next, but this variability did not affect our results, which are presented and analysed in terms of relative rather than absolute numbers of eggs laid.

To measure the oviposition site preference of the different populations, young flies were collected from the cages and put into test cages at a density of 25 females and 25 males per cage. Several cages (usually three) were set up for each test. These cages were the same as the cages used in the selection procedure itself. They were incubated in the dark for up to three days at 26 °C. Often the period was shorter, since sufficient eggs might be laid in 15 hours or overnight. The vials were then removed from the cages, dried yeast added to each, and the flies were allowed to reach the pupa stage before being counted. The number of pupae

found in the vials of sugar medium, expressed as a percentage of all pupae counted, provided our measure of oviposition site preferences.

The data on pupae numbers obtained as described above were analysed in two different ways. Differences between the allopatric lines were tested using the G-test (Sokal & Rohlf, 1981) on the data from each testing period separately. The progress of selection in the individual lines was tested using the test for a linear trend on proportions described by Snedecor & Cochran (1978). For both tests, the appropriate variable is the total number of pupae found in the vials of any one medium.

Preferences displayed by females in the sympatric lines were also measured directly. For any particular cage the number and phenotypes of all the flies developing from all the eight vials in the cage were recorded. Orange-eyed flies develop from eggs laid by females of either eye colour, and these were ignored. The relative numbers of red-eyed and white-eyed flies laid on the two media will give an indication of the oviposition preferences of the female flies in a cage. If oviposition is random, the numbers of flies counted should show no association between eye-colour and oviposition medium. Such associations were identified using the G-test.

(vii) *Dehabituation and habituation of flies*

Some of the tests of oviposition site preference were carried out following the 'dehabituation' of the flies tested. This was done by taking flies from the line being tested and allowing them to lay eggs in bottles containing the standard laboratory medium. When flies from the sympatric cages were being tested, virgin flies of each eye colour were collected separately and mated with males of the same eye colour. Flies from eggs laid in the bottles were then either put into fresh bottles of standard medium to produce a further generation or used directly for oviposition preference tests as described above. In what follows, we shall use the phrase 'normal flies' to refer to flies which have been taken directly from the selection cages.

The converse process was also examined. Flies from the original unselected Leicester 1 population were taken through one generation in vials containing either the sugar or the potato medium, and the oviposition preferences of the progeny were tested as described above. The data were analysed using the analysis of variance. Within any cage there was great variability in the number of pupae counted in each tube, and the same was true between replicate cages. The variable used was the number of pupae found in each vial, and the relevant contribution to the 'total sum of squares' was that of the medium-by-line interaction. A significant contribution by this component implied significant differences in oviposition preference between females raised on the two media.

(viii) *Testing reproductive isolation*

Females removed from the population cages were allowed to lay eggs on standard medium, and the flies which developed from these eggs were used to test reproductive isolation. This was done in either of two ways.

1. *Mass mating.* Virgin females and males were collected and aged for three days in vials containing the standard medium. These flies were then placed in half pint milk bottles containing the standard medium, at a ratio of one female to two males, and using equal numbers of red-eyed and white-eyed flies. Approximately 300 flies were placed in each bottle. The bottles were kept in the dark at 26 °C for three hours, after which the females were removed and each was put into a separate vial of standard medium. The eye colour of the male with which she had mated was deduced from the eye colour of her progeny. There was no evidence of multiple mating in these tests or those described below. Departures from random mating were detected using the χ^2 test. Since each

female can mate with either of two types of male, and these males are equally numerous, the appropriate null hypothesis to be tested is that there are equal numbers of homogametic and heterogametic matings.

2. *Individual choice.* Flies were raised, collected and aged as for the mass mating method. Two males, one of each eye colour, were placed in an empty vial and when they had recovered from CO₂ anaesthesia a single virgin female was introduced. The vial was plugged to leave a cylindrical space approximately 2 cm in diameter and 5 cm long. The vial was placed in the dark at 26 °C. After three hours over 70% of the females had mated, and each was placed in a separate vial of standard medium to lay her eggs. As before, the eye colour of the male with which she had mated was deduced from the eye colour of her progeny. The numbers of females of each eye colour were approximately equal.

Departures from random mating were detected using the χ^2 test as before, and where appropriate the amount of reproductive isolation was calculated using

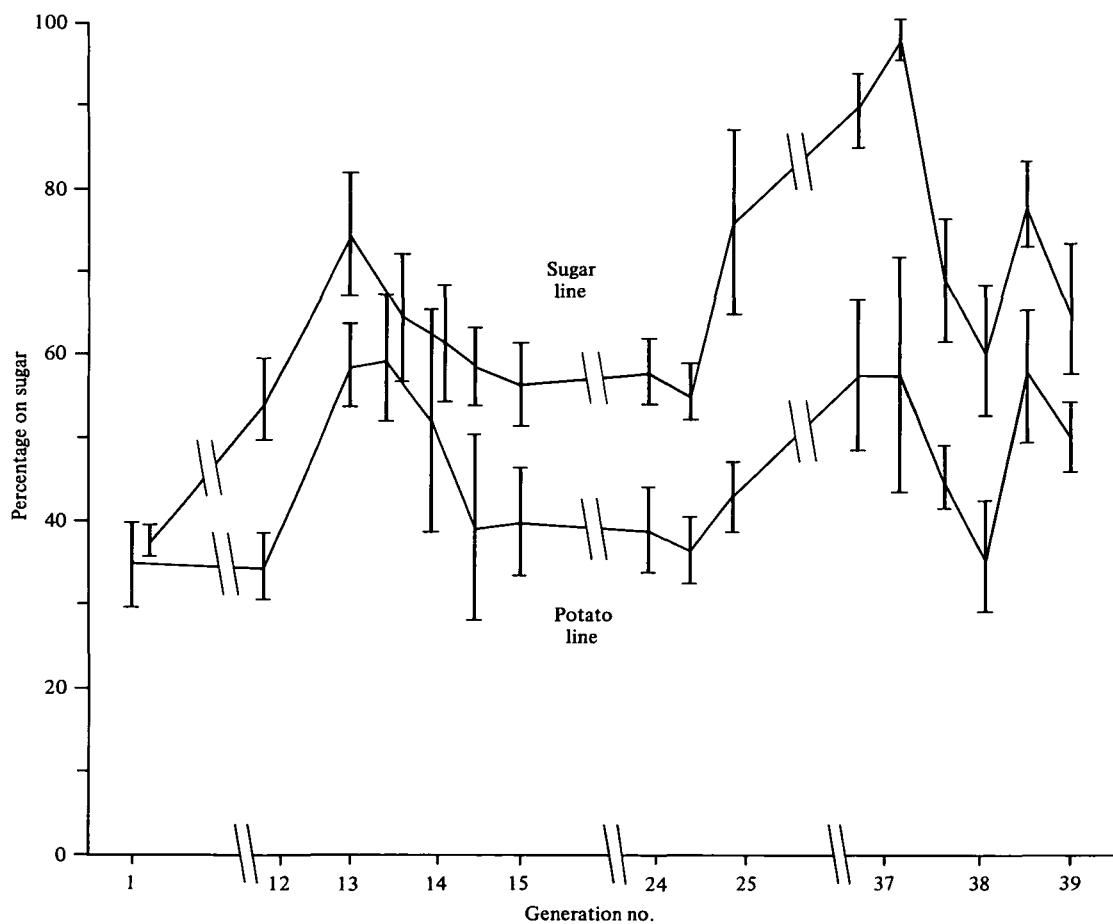


Fig. 1. Progress of selection for oviposition preference in the allopatric lines. Flies were taken directly from the selection cages. Time is measured as approximate generation number, assuming a generation time of two weeks. Preference is expressed as the percentage of pupae to be found on the sugar medium. The upper points are the average of the two sugar-selected lines, and the lower

points the average of the two potato lines. In most cases the data from six test cages were used to fix the points. The error bars are estimated from the range (see text). For the four lines RS, WS, RP and WP the regression coefficients are 0.644 (s.e. = 0.027), 0.770 (s.e. = 0.026), 0.193 (s.e. = 0.027) and -0.050 (s.e. = 0.027) respectively. The units are percentage points per generation.

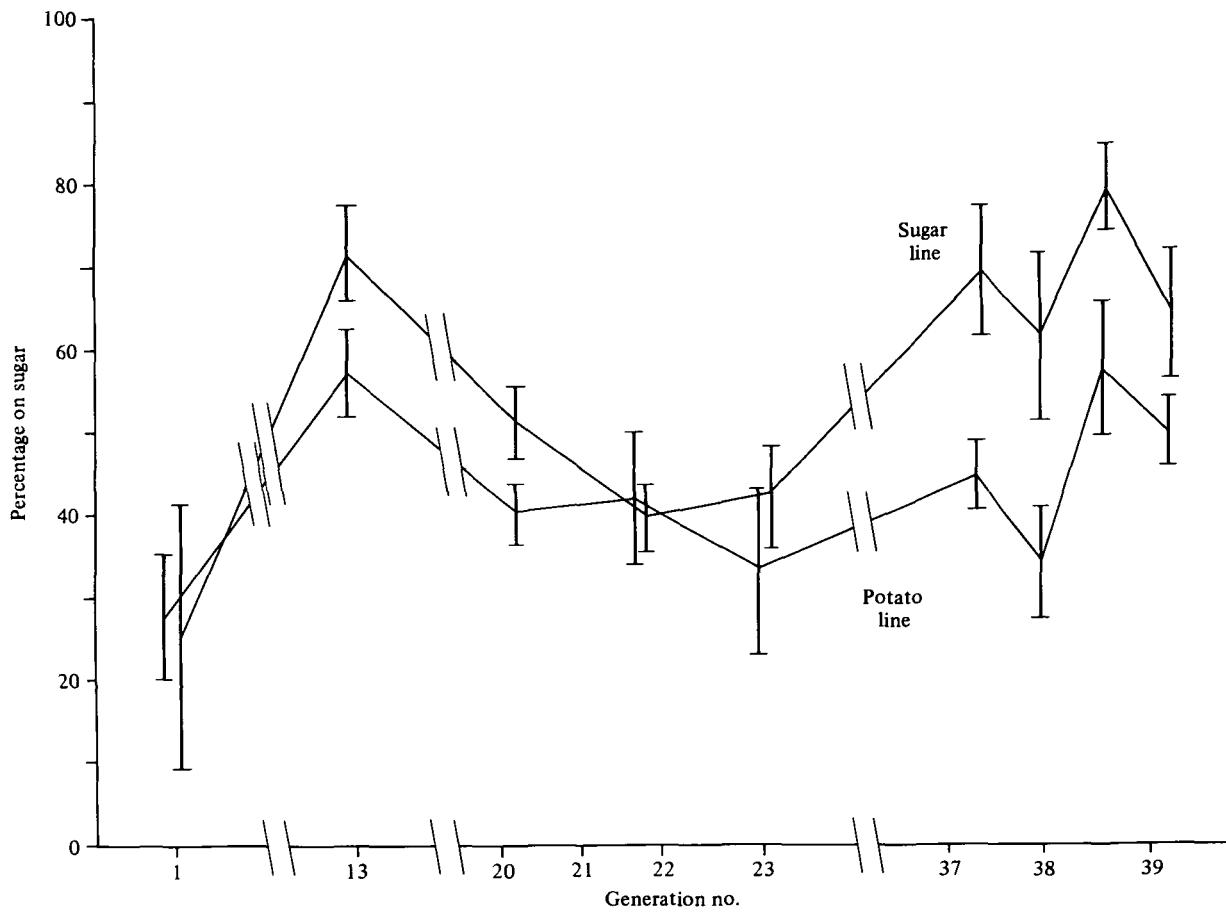


Fig. 2. Preferences displayed by dehabituated flies from each of the four allopatric lines. The results are displayed

as in Fig. 1. Data from the last four sampling times were analysed using the G-test (see text).

the Levene joint isolation index (Ehrman and Petit, 1968, Henderson and Lambert, 1982).

3. Results

Components of fitness such as fecundity, growth rate, and egg to pupa viability have been investigated by Bird (1984). All these were sufficiently invariant between lines, eye-colour genotypes and oviposition medium to exclude them as explanations of the differences described below. In particular, they showed that relative pupa numbers provided an accurate measure of oviposition preference.

(i) Allopatric populations – preferences

At the start of the experiment in March 1982 the lines had exhibited no difference in their choice of oviposition site. After selection was begun, the lines diverged quite rapidly in oviposition preference. Figure 1 shows percentage preference for the sugar medium plotted against approximate generation number. The figure shows a clear divergence in oviposition preference between the pairs of lines designated as 'potato' and 'sugar' lines. Each point on the graph represents the mean preference for the sugar medium of the two lines in question, and is based on

percentage preferences calculated from several separate cages, usually a total of six. The error bars represent the approximate standard error of these means, and are unweighted estimates calculated from the range (Snedecor and Cochran, 1978).

Although there is a large degree of secular variation which is common to both pairs of lines and which probably reflects differences between batches of medium, the figure shows that the lines had diverged quite markedly by the end of the experiment. The G-test was performed on the data corresponding to each of the sampling times in Fig. 1, and in every case yielded a significant 'line by medium' interaction. In other words, the oviposition behaviour of the flies, regardless of their eye-colour genotype, depended on the selection regime. The figure shows a difference in the response to the two types of selection. This is summarized in the legend to Fig. 1, where we give the regression coefficient calculated for the trend in proportions in each case. The two sugar lines show roughly the same response to selection, whereas no overall response is seen for the potato lines. For these lines, the coefficients are very small. One shows a response in the 'right' direction, and one in the 'wrong'.

The rapid early divergence in oviposition site preference raises the question: to what extent do flies become habituated to a particular medium? In other

Table 1. Oviposition preference in sympatric lines, tested directly by counting red-eyed and white-eyed flies as described in the text

| | | Eye colours | | | | | | | |
|------|--------|-------------|-------|-----|-------|-----|-------|-----|-------|
| Cage | Medium | Red | White | Red | White | Red | White | Red | White |
| 4S1 | Potato | 19 | 44 | 81 | 366 | 5 | 122 | 50 | 133 |
| | Sugar | 113 | 146 | 29 | 104 | 30 | 179 | 26 | 18 |
| 4S2 | Potato | 3 | 26 | 110 | 417 | 20 | 214 | 63 | 162 |
| | Sugar | 37 | 22 | 84 | 83 | 91 | 154 | 27 | 49 |
| 4P1 | Potato | 65 | 9 | 464 | 99 | 287 | 8 | 267 | 29 |
| | Sugar | 125 | 82 | 100 | 61 | 213 | 110 | 136 | 37 |
| 4P2 | Potato | 64 | 9 | 204 | 112 | 304 | 26 | 232 | 55 |
| | Sugar | 106 | 85 | 107 | 65 | 139 | 35 | 149 | 42 |

| | | Population | | | |
|------------------------------------|------|------------|-------|-------|-------|
| G-component and degrees of freedom | | 4S1 | 4S2 | 4P1 | 4P2 |
| Day by medium | (3) | 398.2 | 141.6 | 272.2 | 121.5 |
| Day by eye colour | (3) | 104.6 | 18.4 | 36.5 | 103.0 |
| Eye colour by medium | (1) | 27.3 | 102.2 | 144.5 | 27.7 |
| 3-way heterogeneity | (3) | 3.2 | 33.0 | 30.6 | 15.1 |
| Total | (10) | 533.3 | 295.2 | 483.8 | 267.4 |

(a) Numbers of red-eyed and white-eyed flies emerging in vials containing each of the two media. The test was performed four times for each cage. (b) The components of the G-statistic (Sokal & Rohlf, 1981).

words, do females tend to lay eggs on the same medium on which they themselves developed?

When flies from the original Leicester 1 stock were habituated as described above, females raised on the sugar medium laid 5.5% more of their eggs on sugar than did females raised on the potato medium. Analysis of variance showed this difference to be significant at the 5% level. This agrees with the findings of Jaenike (1982, 1983), who showed that adult (but not larval) experience of a given medium could in some cases increase a female's propensity to lay on that medium.

In a similar way, when flies from lines which had been selected for preference for a particular oviposition medium were dehabituated, the extent of the divergence between the sugar and potato lines was reduced. The data on 'dehabituated preference' over the period July 1982 to March 1983 are presented in Fig. 2. The G-test was performed on the data from the last 4 sampling times, and in every case a significant 'line by medium' interaction was found, indicating that a heritable change in the oviposition site preferences of the lines had been achieved.

(ii) Sympatric populations – preferences

Each of the four sympatric populations was tested for divergent oviposition preference by counting eye colours as described above. The count was performed

on four separate occasions in a space of just over two months. The data were analysed using the G-test, the results of which are shown in Table 1. The test revealed differences in the overall acceptability of the two media over this period, and also in the productivity of the two eye-colour types. Nevertheless, the results for each of the four cages show highly significant excesses in the numbers of red-eyed flies found on the designated medium, suggesting that the flies in any one of these cages could be considered as two populations between which gene flow had not prevented the generation of genetically determined differences in oviposition site preference.

In order to check for the possibility of habituation affecting the reality of these results, 'dehabituating tests' were performed on these flies. Three separate tests were performed on each population, using both normal and dehabituated flies. The results from each population are displayed in Fig. 3. They were analysed using the G-test, and results are presented in Table 2. The tests show differences in total productivity between the subpopulations in each cage, and also in the overall acceptability of the two media. They also show heterogeneity between the three tests, which were done on different days. Nevertheless, for the cages 4S1 and 4S2 each of the six trials showed a large significant excess of eggs laid on the 'correct' media, suggesting that a heritable difference in oviposition preference has been generated in these two cages. It will be noted

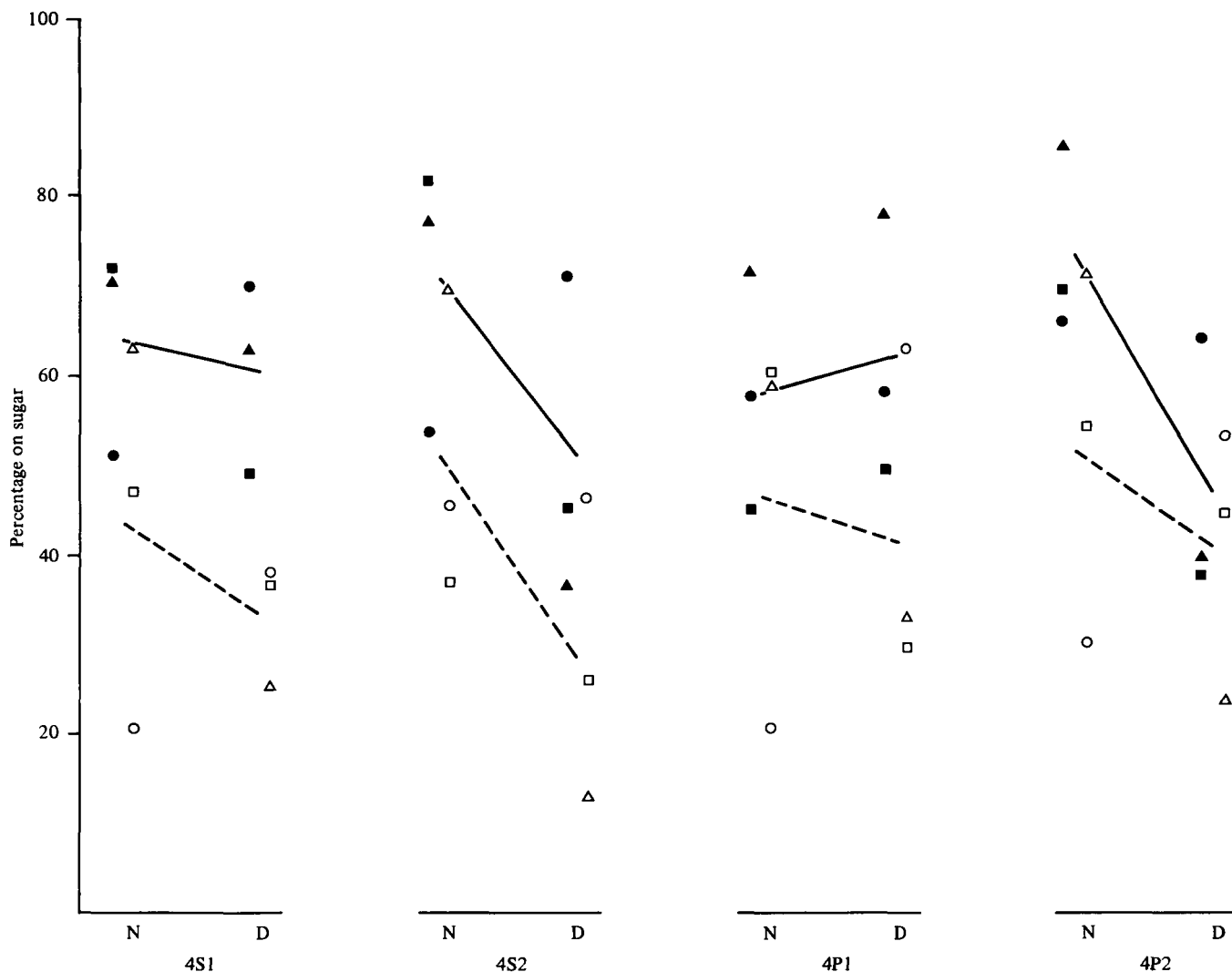


Fig. 3. Comparison between normal (N) and dehabituated (D) flies from the sympatric populations. ('Normal' flies are flies taken direct from the selection cages.) Points show the percentage of pupae found on sugar. For each population results are displayed as follows. Closed symbols: eye colour favoured on sugar; open symbols: eye colour favoured on potato. First set: normal flies; second set: dehabituated flies. The three

symbols, triangle, square and circle, represent the three days on which tests were performed on the normal flies, and the three (different) days on which tests were performed on the dehabituated flies. Each point on the diagram represents the data from one cage. The lines join the mean values for normal and dehabituated flies. Solid line: eye colour favoured on sugar; broken line: eye colour favoured on potato.

Table 2. Oviposition preference in sympatric cages tested after dehabituating in choice cages as described in the text.

| G-component and degrees of freedom | | Population | | | |
|------------------------------------|-----|------------|--------|-------|-------|
| | | 4S1 | 4S2 | 4P1 | 4P2 |
| Day by medium | (2) | 87.7 | 666.0 | 363.7 | 319.9 |
| Day by eye colour | (2) | 76.1 | 160.1 | 63.0 | 120.9 |
| Eye colour by medium | (1) | 398.9 | 505.4 | 107.7 | 23.0 |
| 3-way heterogeneity | (2) | 79.5 | 37.9 | 185.5 | 134.6 |
| Total | (7) | 642.2 | 1369.4 | 719.9 | 598.4 |

The results are summarized in Fig. 3. The table shows the components of the G-statistics.

that the divergences in oviposition preference are similar to those found for the allopatric lines.

The results for cages 4P1 and 4P2 are equivocal. Although the pooled results show an overall tendency to lay preferentially on the designated media, the day-to-day heterogeneity is very much greater. Indeed, for each cage one of the three trials gave results which demonstrated a small, but significant preference in the 'wrong' direction. For this reason we are less ready to claim that reproductive isolation has been generated in these cages.

(iii) Allopatric lines – reproductive isolation

Preliminary experiments had shown that males which had developed on the potato medium were generally more successful in courtship than males from the sugar medium. This unexpected result was independent of the source or eye colour of the males, and was likewise independent of the females used. For this reason all flies used in these tests were raised on standard medium. When tested in this way the original Leicester 1 stock had been shown to mate approximately at random.

The allopatric populations were tested in two pairwise comparisons. Flies from the RP cage were tested against flies from the WS cage, and those from RS against those from WP. When the mass mating test was used, the first comparison gave 127 heterogametic and 126 homogametic matings, and the second gave 125 heterogametic and 147 homogametic matings. In both cases white-eyed males were considerably more successful in mating, but neither result shows a significant degree of assortative mating.

In the mass mating test, many different factors will operate to determine the mating success of flies. If the individual choice method is used, male competition and possibly female choice become the major factors. To check for the operation of these factors, the two

comparisons were repeated using the individual choice method. Three such tests were run, in March, May and June of 1983. The results are shown in Table 3. The first pair of cages, RP and WS, which in the mass mating test showed no isolation, gave a similar result in the individual mating test. The second pair, RS and WP, which in the mass mating test gave a non-significant excess of homogametic over heterogametic matings, gave significant excesses in two out of three tests, and when the results of the three tests are pooled, they are seen to be homogeneous, and the overall excess is significant at the 1% level. Table 3 also gives the value of the Levene joint isolation index for each of the three tests and for the pooled data.

These results lead us to conclude that selection for oviposition preference, which has been successful in producing a heritable change in this character, has been at least partially successful in producing a heritable change in mating behaviour.

(iv) Sympatric lines – reproductive isolation

The extent of any reproductive isolation which might have developed between flies of the two eye colours in each of the sympatric cages was measured using the individual choice method. Red-eyed flies and white-eyed flies were collected from a cage, allowed to produce the next generation on standard medium, and tested against each other as described above. This was repeated for each of the four cages. Table 4 summarizes the results of the tests. In all cases more homogametic than heterogametic matings were recorded, but in only one case was the difference significant. Since the combined data do not yield a significant excess, and a heterogeneity test shows that the four results are internally consistent, the data provide no evidence for assortative mating within the sympatric cages.

Table 3. *Reproductive isolation between allopatric lines tested by individual choice method.*

| Cages | Date | Homogametic matings | Heterogametic matings | χ^2 | ZI |
|---|-------|---------------------|-----------------------|----------|---------------|
| RP, WS | March | 72 | 73 | 0.01 | 1.108 ± 0.192 |
| | May | 93 | 77 | 1.50 | 1.129 ± 0.169 |
| | June | 129 | 134 | 0.10 | 0.987 ± 0.124 |
| RS, WP | March | 88 | 70 | 2.05 | 1.258 ± 0.181 |
| | May | 100 | 69 | 5.69 | 1.474 ± 0.193 |
| | June | 284 | 237 | 4.24 | 1.197 ± 0.096 |
| (Total χ^2 for RS, WP = 11.98 with 3 d.f.) | | | | | |
| RS, WP (Total) | | 472 | 376 | 10.87 | 1.255 ± 0.077 |
| (Heterogeneity χ^2 = 1.11 with 2 d.f.) | | | | | |

Each of the two pairs was tested on three occasions. 'ZI' is the Levene joint isolation index. Its standard error is shown in each case. No isolation was found between the first pair tested. For the second pair, the table shows the results of the separate tests, the pooled result and the heterogeneity value of χ^2

Table 4. Reproductive isolation between sympatric lines tested by individual choice method

| Cages | Homogametic matings | Heterogametic matings | χ^2 | ZI |
|---|---------------------|-----------------------|----------|-------------------|
| 4S1 | 188 | 183 | 0.07 | 0.978 \pm 0.105 |
| 4S2 | 250 | 243 | 0.10 | 1.027 \pm 0.092 |
| 4P1 | 195 | 151 | 5.59 | 1.297 \pm 0.126 |
| 4P2 | 305 | 303 | 0.01 | 1.037 \pm 0.084 |
| (Total χ^2 5.77 with 4 d.f.) | | | | |
| (Total) | 938 | 880 | 1.85 | |
| (Heterogeneity χ^2 3.92 with 3 d.f.) | | | | |

Each of the four cages was tested once, as described in the text. The results are presented as for the allopatric cages.

4. Discussion

Our results indicate that it is possible for artificial selection to produce genetic divergence in oviposition behaviour in *D. melanogaster* females, and that this divergence can be generated when selection is performed under either allopatric or sympatric conditions.

The allopatric experiments show the response to be asymmetric, the observed effect being an increased tendency to lay on the sugar medium. Our results also suggest that reproductive isolation could arise as a by-product of such selection. In the case of the allopatric lines, where there was no possibility of gene flow between the flies selected for the two oviposition sites, a significant degree of isolation between one pair of allopatric lines was generated. Indeed, it is interesting to note that our value for the Levene joint isolation index in this case is at least as large as any of those presented by Henderson & Lambert (1982) for tests between *Drosophila* populations from all over the world. On the other hand, in the sympatric lines, in which there was the possibility of some gene flow in each generation, no isolation was generated, in spite of the success in establishing heritable oviposition differences in two cages out of four.

(i) Oviposition site preference

The selection experiments reported here were designed with an ecologically realistic model in mind, that of an organism with overlapping generations occupying a habitat in which the choice of two oviposition sites is permanently available. Our results suggest that when selection favours the choice of one of these over the other (for whatever reasons), a heritable change in the choice of site displayed by the female could become established.

The selection pressures imposed in our experiments differ from those imposed in selection for quantitative characters, in that they favour in an absolute way one of two mutually exclusive characteristics. If selection

is successful in the allopatric lines, then as it proceeds the intensity of selection would be reduced, and would indeed become zero if oviposition site choice became absolute. The intensity of selection will also be influenced by habituation effects. If habituation was complete, and females always laid their eggs on the medium on which they themselves had been raised, the selection technique described here would not have been possible. The only genetic divergence between populations would be the result of random drift.

In the case of the sympatric lines, our success in selecting for oviposition site preference is similar to that of Pimentel, Smith & Soans (1967). In their experiments gene flow was kept low by the design of the cages used, a design which meant that migration between the sympatric groups was physically limited. In our sympatric cage experiments, there are two separate selection pressures involved, one for oviposition site preference, and the other for reproductive isolation, and the situation is therefore different from the allopatric case. As explained above, there was some gene flow, for genes not on the second chromosome, between the two groups of flies in a case. This gene flow will be reduced if some females happen to be still virgins when they are returned to the cages. Under our selection regime, most females had mated before they were returned to the cages. Thus the genetic effects resulting from artificial selection will always be opposed by gene flow.

The consequences of habituation are similar to those in the allopatric lines, and if habituation were complete selection for oviposition site preference would again be impossible.

(ii) Reproductive isolation

In the allopatric lines selection was entirely for oviposition preference, yet during the course of selection reproductive isolation nevertheless appeared. This could have been due to alleles responsible for features of reproductive isolation 'hitch-hiking' with linked alleles for oviposition preference, or to random drift

of allele frequencies in the separate populations. The latter is unlikely to be true since, if it were, the effect would be a common phenomenon in laboratory populations.

Our results are qualitatively similar to those of Kiliyas, Alahiotis & Pelecanos (1980). In their experiments separate populations were maintained for several years under conditions of either low temperature and low humidity or high temperature and high humidity. They found that significant isolation appeared between populations raised in the two environments. However, their two environments also differed in lighting regime. Harper (1983) demonstrated that if two populations were entrained to diurnal rhythms which were out of phase, reproductive isolation would be observed. Such isolation would therefore be environmentally generated, rather than genetically determined. All our populations were maintained together and so the evidence for a genetic component in the isolation between our populations is stronger.

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