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A test of sexual isolation in *Drosophila*

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1. INTRODUCTION

Little information exists with respect to the occurrence within populations of genetic variation which can give rise to intra-specific sexual isolation. Knight, Robertson & Waddington (1956) and Crossley (1963) established partial sexual isolation between ebony and wingless *vestigial* flies in *Drosophila melanogaster*. However, since wings play a major role in the courtship of the species and wingless individuals do not occur in the wild, this example has little relevance to natural situations. More recently Thoday & Gibson (1962) have reported evidence of assortative mating in the same species between individuals differing in bristle number and suggested that sexual isolation can be established readily enough to promote sympatric speciation.

As a result of recent studies of adaptation of populations of *D. melanogaster* to a new diet (Robertson, 1966), peculiarly favourable material became available for putting some of these ideas to the test. The populations were adapted to a new diet containing the chelating agent, EDTA, which reduces body size, lengthens development time and lowers survival, according to the concentration, as Steffensen (1957) first showed. The populations were all derived from the Pacific cage population. One of the strains adapted to the EDTA medium in a population cage was chosen for the experiments described here. On media with 0·005–0·01 M EDTA, this strain grows faster to a larger size and has a higher survival than the original Pacific population. Under crowded, competitive conditions on the EDTA medium, the superiority of this strain is very great. But, when EDTA is not added to the medium, under crowded conditions the situation is reversed and it is the EDTA-adapted strain which has the slower growth and smaller body size. The F_1 of the cross between the original and the EDTA-adapted population is roughly intermediate and has lower performance on medium with or without EDTA than the strain adapted to one or the other type of diet.

Genetic analysis showed that adaptation to EDTA had involved changes in all major chromosomes and substantial interaction between non-homologous chromosomes. For example, substitution of the third pair of chromosomes from the original population for their homologues, in the background of the EDTA-adapted strain, caused complete sterility and also lethality at higher EDTA concentrations.

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Since the differences in reaction to environments and in genetic composition were so well defined, these two populations were ideally suited for an attempt to establish reproductive isolation in the following way. Pairs of population cages were placed in communication by glass tubes 3 in. long and either 1 in. or ½ in. in diameter, so that flies could move from one compartment to the other. One of the cages in each pair was provided with medium without EDTA and also without additional dried yeast, to promote more competitive conditions; the other was supplied with medium containing EDTA at a level of 0·01 M which is an unfavourable concentration for the growth of the original Pacific population. A large sample of flies from the original and the EDTA-adapted population was introduced into cages supplied respectively with either EDTA-free medium or with medium containing EDTA. The only communication between the members of each pair of cages was via the glass tube. Three pairs of such cages were set up at the same time, two with the wider (Nos. 1 and 2) and one with the narrower (No. 3) connexion. The cages were kept at 25°C. for many generations during which the appropriate food medium was replaced regularly at the same time in each cage. The number of flies in the cages was not counted but periodic inspection showed thriving populations in all cases. Flies were observed moving between the two compartments but the rate of immigration appeared to be low.

After about fifteen and twenty-five generations, samples of eggs were collected from each cage and cultured on media with or without EDTA, generally in eight replicate vials, to compare the performance of the connected sub-populations with each other and also with that of the original Pacific and the EDTA-adapted populations, which were maintained under their usual conditions. Body size was measured as 3 $\log_e$ thorax length in 1/100 mm. and larval period as $\log_e$ development time in days, minus the average pupal period of 4·3 days. For each category 50–60 flies were measured, while 100–150 were scored for development time; only females were compared. The deviations between means have been multiplied by 100 and therefore approximate to percentage differences.

At about generation 20 a test of assortative mating was carried out as follows. Eggs from each cage were set up on the corresponding medium, sexes were separated at eclosion and the flies were aged for 2 to 3 days. Flies from one of each pair of connected cages were lightly etherized and marked on the thorax with a spot of quick-drying paint; flies from the other cage were also lightly etherized to ensure similarity of handling procedure. One or two days later, marked males and females and an equal number of unmarked flies from the other sub-population of each pair of cages were combined. Generally twenty-five pairs of each type were released into a half-pint bottle. As they mated the pairs were removed by gentle suction and scored.

In addition, artificial selection for assortative mating was carried out. Flies of the original and the EDTA-adapted strain were cultured on their appropriate medium and one or other type was marked and handled as above. Equal numbers were combined and parents of successive generations were chosen exclusively from matings between flies from the same population. Considerable variation is

often encountered in the time taken to mate in tests of this kind. To avoid possible bias, due to change in average speed of mating, the parents were chosen from the total number of assortatively mated pairs so that their average time to mating was the same in the two series in any generation. Generally about forty to fifty pairs of each type were tested and about six to eight pairs of parents were combined and kept for about 2 days after which eggs were collected from the mass mating and set up on the appropriate medium.

2. RESULTS

(i) *Comparisons between sub-populations*

Table 1 shows the deviations in body size and larval period between flies from each cage and the corresponding parent population on the usual medium, without EDTA, and also on media with 0·005 and 0·01 M EDTA after approximately fifteen and twenty-five generations respectively. The deviations indicate how far each derived population has changed due to immigration from the other cage. Table 2 shows the difference in average performance between flies derived from either cage of each pair and provides a measure of genetic differences between them. Naturally, corresponding differences between the same populations vary according to the nature of the diet.

Table 1 shows that each sub-population had diverged from the parent population. Thus, in the comparisons with the original Pacific population on medium with EDTA, the deviations for development time are negative, i.e. the sub-populations grew much faster than the parent population. In the corresponding comparisons with the EDTA-adapted population, the deviations for development time are positive, i.e. the sub-populations were not so well adapted as the parent population. Evidently there was substantial convergence between the sub-population due to immigration. In the later test the deviations from the parent populations show a greater divergence from the original Pacific population, but a diminished divergence from the EDTA-adapted population. This may mean that there had been an unequal spread of genes from one population to the other, due to differences in rate of migration or, more likely, to natural selection of a new gene combination which conferred greater tolerance to the alternative diets.

Table 2 shows that in spite of the convergence there were well-defined differences between the sub-populations for each pair of cages at the time of both tests. Oddly enough flies from the cages connected by the narrow bore tubing (No. 3) showed the least difference. For the other pairs, there were substantial differences, especially evident in the duration of the larval period on the EDTA medium.

(ii) *The mating test*

A test of assortative mating was carried out at approximately generation 20, i.e. at a time when, in spite of substantial convergence, there was nevertheless clear evidence of genetic differences between the sub-populations. The mating tests

Table 1. *Deviations from parent strains on alternative diets*— × *100*

	Generation 15						Generation 25					
	Zero EDTA		0·005 M EDTA		0·01 M EDTA		Zero EDTA		0·005 M EDTA		0·01 M EDTA	
Sub-populations	P	E	P	E	P	E	P	E	P	E	P	E
	Body size (3 $\log_e$ thorax length)											
1	−3·0	−7·3*	5·6	0·7	−23·0**	0·1	7·2**	0·3	9·3**	−0·7	—	−2·4
2	−2·6	−0·7	0·8	4·3	4·6	0·7	4·3*	−3·8*	8·1**	0·9	—	−0·9
3	−4·5	−3·9	8·8*	0·7	7·8*	−3·8	4·7*	−3·2*	4·6	−1·4	—	−4·6
	$\log_e$ larval life											
1	1·4	2·1	0·7	16·5**	−7·0	14·3**	−8·3*	−0·7	−47·6**	2·1	—	−2·0
2	0·8	0·9	−5·1	13·8**	−21·0**	20·8**	−11·0*	−0·2	−49·7**	11·7*	—	−7·4*
3	4·5	−7·4*	−27·8**	15·0**	−28·6**	21·3**	−11·4*	1·0	−54·6**	14·8*	—	8·2

For body size positive and negative differences indicate that the sub-populations are larger or smaller than the parent population and, for the larval period, that the sub-populations develop faster or slower than the parent population.

P and E refer respectively to the sub-populations derived from either the Pacific or the EDTA-adapted population.

* and ** indicate significance at the 0·05 and 0·01 level of probability.

Table 2. *Differences between sub-populations on different diets— × 100*

Sub-populations	Generation 15			Generation 25		
	Zero EDTA	0·005 M EDTA	0·01 M EDTA	Zero EDTA	0·005 M EDTA	0·01 M EDTA
	Body size (3 $\log_e$ thorax length)					
1	7·3*	−3·5	−28·0**	3·5*	0·3	−0·9
2	0·0	−3·4	−1·0	4·7*	−2·5	0·5
3	1·3	−0·3	6·7	4·5*	−3·7*	1·3
Difference between parent populations, Pacific–EDTA	3·0*	−9·6**	−4·8*	−3·4*	−9·7**	—
	Log_e larval period					
1	5·2	28·7**	28·5**	7·9*	16·6**	19·0**
2	3·4	25·6**	8·0	4·3	9·7*	2·7
3	7·4	1·7	0·0	3·1	1·7	12·1*
Difference between parent populations, Pacific–EDTA	−4·5*	44·5**	49·8**	15·8**	71·1**	—

The mean values for the sub-populations from cages with the EDTA medium are subtracted from the corresponding values for the sub-populations from cages with the EDTA-free medium.
In the test at generation 25, medium with 0·01 M EDTA was lethal for the Pacific individuals.
* and ** indicate significance at the 0·05 and 0·01 level of probability.

were carried out by combining twenty-five marked males and females with an equal number of unmarked individuals of either sex. Several such replicated trials were carried out in each test, but, since there was no evidence of heterogeneity in the distribution of the types of mated pairs, the data from all replicates have been combined. In each test there were four alternative combinations with respect to origin and sex. The departures from random combinations have been tested by χ^2, for which there is one degree of freedom per test.

Table 3 offers no evidence in any of the tests for a statistically significant departure from random distribution, i.e. the evidence for assortative mating between flies from the same sub-population was nil.

Table 3. *Test of departure from random mating*

Paired sub-populations	Number of matings			d.f.	χ^2	p
	Assortative	Disassortative	Total			
1	83	106	189	1	2·62	> 0·05
2	53	59	112	1	0·21	> 0·05
3	54	53	107	1	2·70	> 0·05

(iii) *Selection for assortative mating*

Figure 1 shows the result of fourteen generations of selection for assortative mating. The data are plotted as deviations from the 50% assortatively mated pairs expected with random mating. In spite of their prolonged genetic isolation and considerable genetic divergence there was no evidence of isolation between the

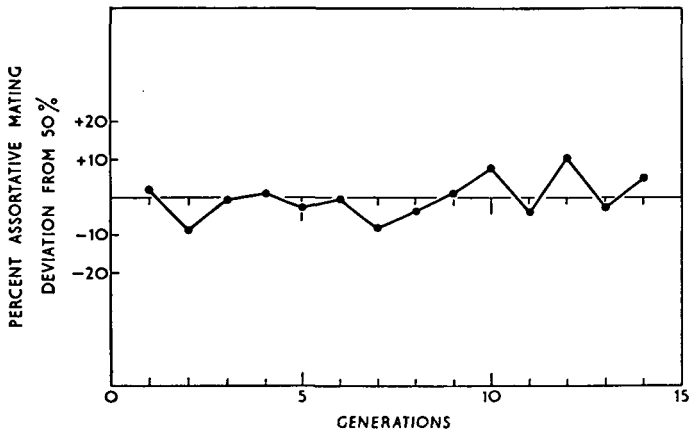

Fig. 1. The effects of selecting for sexual isolation between the Pacific and the EDTA-adapted cage population. Flies of a different origin were distinguished by paint spots. Equal numbers of males and females of each type were allowed to mate and then scored for type of mating. Parents in successive generations were drawn exclusively from matings between flies of the same origin.

two parent strains at the start of the experiment nor any indication that selection had succeeded in establishing isolation. The positive deviations at generations 10 and 12 are statistically insignificant; p exceeds 0·1 in each instance.

3. DISCUSSION

These experiments have failed to provide any evidence for the development of sexual isolation in an ecological situation which would seem to favour its occurrence. Such evidence is not incompatible with the experience of Knight *et al.* (1956) or Crossley (1963), who required a major phenotypic difference between the types as well as many generations of selection to establish partial isolation. Thoday and Gibson's (1962) example of isolation between individuals differing in bristle number is apparently exceptional and merits re-examination. In the light of the present and the earlier experiments, sympatric divergence in a species like *D. melanogaster* would appear to be rather improbable.

SUMMARY

1. A test is described for the development of sexual isolation between a wild and a derived population of *D. melanogaster* adapted to a new diet, containing EDTA. Other experiments had shown that adaptation to the new diet involved genetic changes in all chromosomes. Also fitness was reversed on the alternative diets under crowded competitive conditions.

2. In three replicated trials flies from each population were used to establish paired cage populations, supplied with the medium to which each was adapted, and the pairs of cages were joined to allow restricted immigration between them. The experiment was run for about twenty-five generations.

3. After fifteen and twenty-five generations, flies were collected from each cage to provide eggs which were cultured on the alternative diets to determine how far the members of pairs of populations differed from each other and from the foundation population. There were striking differences between the sub-populations and the parent populations, attributable to immigration between the former. Judged by the differences in performance between the sub-populations, genetic differences persisted but these were minor compared with the differences between the parent populations.

4. Tests of preferential mating on the part of flies from paired sub-populations were entirely negative.

5. Fourteen generations of selection for positive assortative mating failed to provide evidence of sexual isolation between the two basic populations, adapted to different diets.

6. From these and other experiments it is inferred that sympatric divergence is improbable in a species like *D. melanogaster*.

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