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Genetic divergence in M. Vetukhiv's experimental populations of *Drosophila pseudoobscura*

1. RUDIMENTS OF SEXUAL ISOLATION*

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1. INTRODUCTION AND MATERIALS

In a series of studies published by M. Vetukhiv (1953, 1954*a* and *b*, 1956*a*, *b* and *c*, 1957; Vetukhiv & Beardmore, 1959; Wallace and Vetukhiv, 1955), he described the longevity, fecundity, and larval competitive ability of F_1 and F_2 hybrids between geographic populations of certain species of *Drosophila*. In most, though not in all cases, the F_1 hybrids were superior to the parental populations in all the parameters examined. In fact, the hybrid populations usually exceeded not only the mid-parent but even the superior parent. Contrasting with this, the F_2 hybrid generation suffers a 'hybrid breakdown'; not only does the F_2 lack the apparent heterosis of the F_1 hybrids but the second generation hybrids tend to fall below the parental populations. These results cannot be accounted for by supposing that the parents crossed were inbred; although the original strains used were kept in laboratory cultures for some generations before the beginning of the experiments, the 'parents' were never single strains but populations obtained by intercrossing all the available strains from a given locality. In other words, care was taken to obtain, in laboratory cultures, as faithful replicas of the natural populations as practicable. It appeared, then, that when the gene pools of two populations diverge, there are likely to arise, in some but not in all of them, genetic systems of a kind which produce heterosis in F_1 hybrids and a genetic breakdown in F_2.

In May 1958, Dr Vetukhiv started a new series of experiments, designed to test the possibility that genetic systems of the above sort may arise not only in natural but also in experimental populations in the laboratory. He arranged six populations, to be kept in the wooden 'population cages' of a model which was widely used in the laboratory of Professor Th. Dobzhansky at Columbia University (Dobzhansky, 1947). Two of these populations were maintained in a constant temperature room at 16° C., two in an incubator or in a constant temperature room at 25° C., and the remaining two in an incubator at 27° C. The founders of these six populations were the same. In order to provide the populations with as much genetic variability as possible, to give selection material to work with, Vetukhiv chose to use as founders

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hybrids from more than forty strains, at least ten from each of the following geographic localities: Mather, California; Bryce National Park, Utah; Ferron, Utah; and Gunnison, Colorado. Each strain was the progeny of a single female, fertilized in nature by presumably a single male, collected in the respective localities by Professor Th. Dobzhansky in the summer of 1950. All strains were examined cytologically by Professor Dobzhansky, and all strains used by Dr Vetukhiv had the Arrowhead gene arrangement in their third chromosomes, and no other chromosomal polymorphisms. The technique of obtaining hybrids was as follows: virgin females from all the strains from the first locality were mated to males from all the strains from the second, and females from the third to males from the fourth, and vice versa (i.e. reciprocal crosses were made to insure equal participation of the gene pools of the four geographic populations). The F_1 hybrids were intercrossed to obtain quadruple hybrids. All these crosses were made in regular culture bottles. When the quadruple hybrids hatched, a group of about 1000 of them, about equally females and males, and derived about equally from all cultures, were placed in a population cage to serve as founders. The cups with food and with eggs deposited on them over a period of a day or two were removed to a population cage without adult flies. Fresh cups were introduced into the cage with the founders, and a new batch of eggs collected. This was repeated six times, so that six population cages were obtained with progenies of the same group of founders. The population cages were then distributed in pairs to the three different temperatures.

After the untimely death of Dr Vetukhiv in June of 1959, the populations were maintained by Mrs O. Pavlovsky, Mrs N. Spassky and, finally, by Mr B. Spassky. The temperature of 27° C. is close to the upper limit at which most strains of *D. pseudoobscura* can be kept in the laboratory for more than a single generation. The population cages at this temperature were at first difficult to maintain, and they had to be given temporary respite by transferring them to 25° C. for a generation or so. Eventually they became reasonably vigorous, enough to be maintained exclusively at 27° C., although the numbers of adult flies in these populations were always smaller than in those kept at lower temperatures.

It is difficult to estimate how many generations intervened between the foundation of the populations and the time when flies were taken from them for the purposes of the experiments described below, except that at 16° C. there were only about half as many generations as at 25° C., and at 25° C., somewhat fewer than at 27° C. It is probably fair to say that in population cages at 25° C., there occur between ten and fifteen generations per year, and that the generations broadly overlap. (See Barker, 1962, for a discussion of the estimation of generation interval in experimental populations of *Drosophila*.)

2. METHOD

On October 6, 1962, when the six population cages were 4 years and 5 months old, samples of about twenty adults were taken from each cage and thereafter subcultured and maintained in uncrowded culture bottles under optimal nutritional

conditions at room temperature. The flies used for these experiments have, accordingly, developed under similar conditions. However, the six population cages and the temperature at which they existed for the 4 years and 5 months before the start of these experiments were:

A and B at 16° C.
C and D at 25° C.
E and F at 27° C.

Virgins of both sexes were collected from cultures of each line, and aged for 4 days at room temperature. (Males were sometimes aged for as long as 5 days.) Flies which were utilized in these experiments were etherized only once—when they were 'sexed'. At this same time, one wing of each of half of the females was clipped slightly on its distal margin for ease of identification. Groups of ten males of one kind with ten females of the same kind, plus ten more females of another cage sample ($10X♂♂ + 10X♀♀ + 10Y♀♀$), were then confined in vials containing food for 2 hours at 27° C. or for $2\frac{1}{4}$ hours at 16° C., the two extreme temperatures at which the population cages were kept. A pilot experiment has shown that these time intervals give about 50% of the females inseminated.

After 2 hours or $2\frac{1}{4}$ hours had elapsed, the entire contents of each vial were etherized and males discarded. Thus, males were never used more than once. The females were then sorted (clipped and non-clipped wings). Then the sperm-storing organs (ventral receptacles and spermathecae) were dissected out in physiological saline and examined for the presence of spermatozoa. The tallies from the following vials were discarded:

(1) those in which fewer than twenty females were alive at the close of the multiple choice experiment,
(2) those in which more than fourteen females (70%) were inseminated, and
(3) those in which fewer than six females (30%) were inseminated.

Too little insemination indicates that the flies were apparently not in optimal condition when placed together. Too much insemination must be eliminated since the experimental scheme does not allow for the scoring of sequence of insemination.

With six populations involved, and taking into consideration two extreme temperatures and reciprocal crosses,

e.g. $10X♂♂ + 10X♀♀ + 10Y♀♀$ *and* $10Y♂♂ + 10Y♀♀ + 10X♀♀$,

twenty-four crucial crosses were tested. Two hundred females were dissected from each cross—one hundred of each of two kinds, making up ten vials, so that a total of 4800 females were dissected: $10 \times 10 \times 2$ (or 200) $\times 24 = 4800$. Of course, more than this number were actually dissected, but some counts had to be excluded from the data (see above).

No females from any one cage sample were consistently clipped. This was rotated so that in any given multiple choice cross, females from a single population were clipped only half the time. These experiments were begun on October 6, 1962, and concluded on May 3, 1963.

3. RESULTS

The data are summarized in Table 1. The isolation indexes were calculated according to Stalker (1942), because the per cent of females inseminated was close to fifty, or was exactly 50% in all instances.

Table 1. *Percentages of females inseminated in different crosses*

Populations Crossed	°C	Homogamic	Heterogamic	Isolation Index	Chi square
A × B	16	56	41	0·155	3·92*
	27	53	35	0·204	5·86**
B × A	16	53	49	0·039	0·18
	27	68	44	0·214	10·74**
C × D	16	47	47	0	0
	27	63	48	0·135	3·97*
D × C	16	62	43	0·181	6·50**
	27	61	37	0·245	10·58**
E × F	16	49	50	−0·010	0
	27	65	43	0·203	8·87**
F × E	16	54	43	0·113	2·00
	27	54	53	0·009	0
A × C	16	55	47	0·078	0·98
	27	60	58	0·017	0·02
C × A	16	51	44	0·073	0·72
	27	50	49	0·010	0
A × E	16	52	39	0·143	2·90
	27	48	41	0·078	0·73
E × A	16	60	43	0·165	5·12**
	27	52	57	−0·045	0·32
C × E	16	72	66	0·043	0·58
	27	69	42	0·243	13·68**
E × C	16	45	47	−0·022	0·02
	27	43	47	−0·033	0·18

* = Significant at the 5% level; ** = significant at the 2·5% level or better.

A Stalker isolation index of +1·00 means only homogamic matings, 0 means that only random matings are taking place, and −1·00 would mean exclusive occurrence of heterogamic matings. There were four only slightly negative isolation indexes but *all* twenty-four joint isolation indexes (simply the mean of the two reciprocal isolation indices) were positive (Table 2). For an excellent review of the value of these, and of other statistics, see Levene (1949).

The chi-squares were computed from two-by-two contingency tables with Yates' correction; each one has one degree of freedom. These are equivalent to a test for the significance of an isolation index. No test of significance for the joint isolation index has been devised.

In Table 1, the per cent inseminated is exactly equal to the number inseminated because in each cross (the rows) there were one hundred possibilities for homogamic

mating as well as one hundred possibilities for heterogamic mating. Thus, in the first cross in Table 1, A × B at 16° C., 56 A females were inseminated and 44 were not; 41 B females were inseminated, while 59 were not.

Table 2. *Joint isolation indexes from different crosses*

Populations crossed	°C	Index
A × B	16	+0·097
	27	+0·209
C × D	16	+0·090
	27	+0·190
E × F	16	+0·052
	27	+0·106
A × C	16	+0·075
	27	+0·014
A × E	16	+0·154
	27	+0·016
C × E	16	+0·011
	27	+0·105

The population indicated first, e.g. A in A × B, denotes the type of male used. Thus, in A × B, A♂♂ were confined with A♀♀ and B♀♀. The third row represents the cross reciprocal to the first, the fourth is reciprocal to the second, etc.

Nine of the chi-squares in Table 1 are significant at, at least, the 5% level; of the twenty-four crosses, nineteen show a greater number of homogamic than of heterogamic matings, four show a greater number of heterogamic matings, and in one cross the number was exactly equal.

4. DISCUSSION

Vetukhiv's (1954*a*) general conclusion from his experiments has been that 'the gene pool of the population of any one geographic region contains ... a variety of coadapted gene complexes ... Natural selection does not, however, adjust the gene complexes in different geographic populations ... for the simple reason that interbreeding of members of geographically remote populations occurs only rarely or not at all ... the genotype of each local population is, in at least some sexually reproducing species, an integrated system which may break down as a result of gene recombination in the hybrids.' How generally valid this conclusion may prove to be is at present an open question. McFarquhar & Robertson (1963) working with geographic races of *Drosophila subobscura* were unable to find any sign either of increased vigor of F_1 hybrids, or of a breakdown in the F_2 hybrids between populations from remote localities.

It is, therefore, especially interesting that, in *D. pseudoobscura*, experimental populations can diverge genetically within only few years time to a point where at least some of them show traces of sexual isolation, and, as will be described in a forthcoming paper by Dr A. Mourad, also of F_1 hybrid vigor and F_2 breakdown. The six experimental populations started by Dr Vetukhiv in 1958 were initially genetically similar, except for the possible differences introduced by sampling; these differences could not have been large because the number of the founders of each population was substantial, close to a thousand. Since the populations were then kept quite separate, with no exchange of migrants between them, the sexual isolation which has developed between some of these populations could not have been a result of selection specifically for such an isolation. It is more tempting to suppose that the sexual isolation arose as a by-product of genetic changes in populations which became adapted to different environments, especially to different temperatures (see the section on the origin of reproductive isolation in Ehrman, 1962). Even this view meets with difficulties; as an inspection of Tables 1 and 2 shows, traces of sexual isolation have appeared, if anything, more often between populations which were kept at the same temperature (populations A and B, C and D) than between populations which lived at different temperatures (no isolation at all between A kept at 16° and C kept at 25°, only a single instance between A and E and between C and E, E being the population kept at 27°).

Genetic divergence evidently takes place between isolated populations kept in similar as well as in different environments (it would, perhaps, be more correct to say in similar or in different macro-environments). Such divergence, due to the combined effects of genetic drift and natural selection, was demonstrated in experimental populations of *D. pseudoobscura* by Dobzhansky & Pavlovsky (1957) and Dobzhansky & Spassky (1962). When populations become different in more and more and more genes, reproductive isolation may arise because the action of many genes is pleiotropic. Some gene differences selected for different reasons, or resulting from random genetic drift, may thus have isolating side effects. These, to be sure, are mere rudiments of reproductive, in our case, sexual isolation. For the completion of the process of isolation, to the point where no hybrids at all would be formed between members of different populations, occasional gene exchange and production of adaptively inferior hybrids would probably be necessary stimuli (Dobzhansky, 1958).

In this connection, comparison of our results with those of Thoday & Gibson (1962) is of special interest. These investigators practiced diversifying (disruptive) selection for high and low sternopleural chaeta numbers on a single population of *D. melanogaster*. As a result of this selection, the population tended to split into two moieties, which, after as few as twelve generations of selection, produced only a limited number of hybrids. Unlike our experiments, in which the populations were kept quite separate, Thoday and Gibson's work seems to demonstrate the origin of reproductive isolation without an antecedent geographical separation. It is nevertheless clear that, as Thoday and Gibson admit, geographical separation facilitates greatly the achievement of complete reproductive isolation.

Given an adaptive infirmity of the hybrids (Vetukhiv, 1954*a*) and an opportunity for their formation (incipient sympatry), natural selection should, if possible, prevent their appearance. An adaptive inferiority of hybrids may, indeed, lower the fitness of both Mendelian populations between which such hybrids arise. Sexual (or psychological or ethological) isolation which makes the mutual attraction between conspecific males and females greater than the attraction between males and females of different species, is perhaps the most efficient means of accomplishing the prevention of gene exchange.

SUMMARY

Weak but statistically significant sexual isolation has been demonstrated among Vetukhiv's six experimental populations of *Drosophila pseudoobscura*, all originally descended from founders taken from cultures of the same hybrids from four geographic localities. These six populations were maintained separately for almost $4\frac{1}{2}$ years and then tested for the existence of sexual isolation. The sexual isolation has arisen in the absence of any selection for isolation, evidently as a by-product of genetic divergence.

Professor Th. Dobzhansky supervised this entire project. Dr A. Mourad participated in many profitable discussions, and Mr G. Carmody kindly checked the mathematics in Table 1.

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