Genet. Res., Camb. (1965), 6, *pp.* 139–146
With 2 *text-figures*
Printed in Great Britain

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

2. LONGEVITY

BY ABD EL-KHALEK MOURAD

The Rockefeller Institute, New York City, and Department of Genetics,
Faculty of Agriculture, University of Alexandria, Egypt

(*Received* 6 *July* 1964)

1. INTRODUCTION

A series of experiments reported by Vetukhiv (1953, 1954, 1956, 1957) have established that F_1 hybrids between populations of *Drosophila pseudoobscura* of different geographic origins tend to be superior to the parental populations in longevity, fecundity and the competitive ability of the larvae in crowded cultures. This apparent heterosis, or luxuriance, does not, however, persist in subsequent generations. In fact, the F_2 hybrids between the populations were, in Vetukhiv's experiments, not only inferior to the F_1, but in some cases to the parental populations. How widespread is this kind of behaviour of hybrids between populations is an open question. McFarquhar & Robertson (1963), working with geographic populations of *Drosophila subobscura*, found no evidence either of an F_1 luxuriance or of an F_2 breakdown.

The present article reports a study of the longevity of the flies from four of the six experimental populations of *D. pseudoobscura* which were started by the late M. Vetukhiv in May 1958, and kept in laboratory population cages since then without interchange or addition of inhabitants. For a description of these populations, see Ehrman (1964). Suffice it to say that all six populations are descended from the same group of about 1000 founders, who were in turn descended from a four-way cross between four geographic populations. Pairs of the populations were then kept at the three temperatures, 16°, 25°, and 27°C. respectively. Ehrman (1964) has demonstrated that these populations have diverged genetically to the extent that a weak but statistically significant sexual (ethological) isolation is observed between some of them.

2. MATERIALS AND METHODS

The temperatures at which the six population cages were kept for a period of about four and half years before the start of this experiment were: A and B at 16°C., C and D at 25°C. and E and F at 27°C. The populations A, C, E, and F, and the hybrids between them, were tested for longevity.

Food cups with pupae that were about to hatch were taken from each cage. Plastic 'chimneys' were mounted on them, and virgin females and males were collected daily. When enough flies were obtained, the crosses necessary for a particular experiment were made on the same day. Since the F_1 inter-population hybrids developed in culture bottles and not in population cages, the parental strains were also reared in culture bottles for one generation. In case of the F_2 inter-population hybrids, their parental strains were reared for two consecutive generations in culture bottles.

From each inter- or intra-population cross, ten replicate cultures were set up. In case of the inter-population hybrids reciprocal crosses were made. Each culture bottle contained 3 or 4 females and an equal number of males, so that the progenies to be tested were reared under uncrowded optimal nutritional conditions at room temperature. Upon hatching, groups of 20 ♀♀ and 20♂♂ of a given kind were placed in ordinary half-pint culture bottles with a synthetic medium devised by Kalmus, and a standard amount of yeast. For each population and for each hybrid series, five such bottles were made; the initial number of the flies was thus 100 ♂♂ and 100 ♀♀.

The longevities were studied at two temperatures, 16°C. and 27°C., 16°C. being an optimal and 27°C. being a sub-lethal temperature for *D. pseudoobscura*. The flies were transferred to bottles with fresh synthetic medium and fresh yeast three times a week at 27°C. and twice a week at 16°C. The numbers of the dead flies of each sex were counted at each transfer. The experiments at 16°C. and 27°C. were begun simultaneously. Whenever the longevity of F_1 or F_2 hybrids was studied, that of the parental strains was examined simultaneously. This is necessary because longevity experiments made at different times with the same strain may give somewhat different values, owing to uncontrollable variations in the food or in other environmental components.

The longevity of the F_1, F_2 and the parents was studied twice in every case in order to ascertain the repeatability of the results. All the crosses that showed a superiority of the F_1 at 27°C., and only one of the crosses that did not show an F_1 superiority were continued into F_2. Unfortunately, the cross A × E which showed F_1 superiority at 16°C. was not examined in the F_2.

3. RESULTS AND DISCUSSION

Tables 1 and 2 show the mean longevities of the different populations and their hybrids at 27°C. and at 16°C. respectively. Figures 1 and 2 illustrate the relationships observed in the form of diagrams; they give, however, the averages of two replicate experiments for both the F_1 and the F_2 at 27°C. and 16°C. Table 3 shows the statistical significance of the observed differences between the mean longevities of the F_1 and F_2 hybrids and the mid-parents values. The tests of significance were made for the females only.

In the experiments conducted at 27°C., the longevity of the F_1 was either not different from the mid-parent value or below it for all the crosses involving

Table 1. *Parents and hybrids at 27°C. Mean longevity (in days) of different populations and their hybrids*

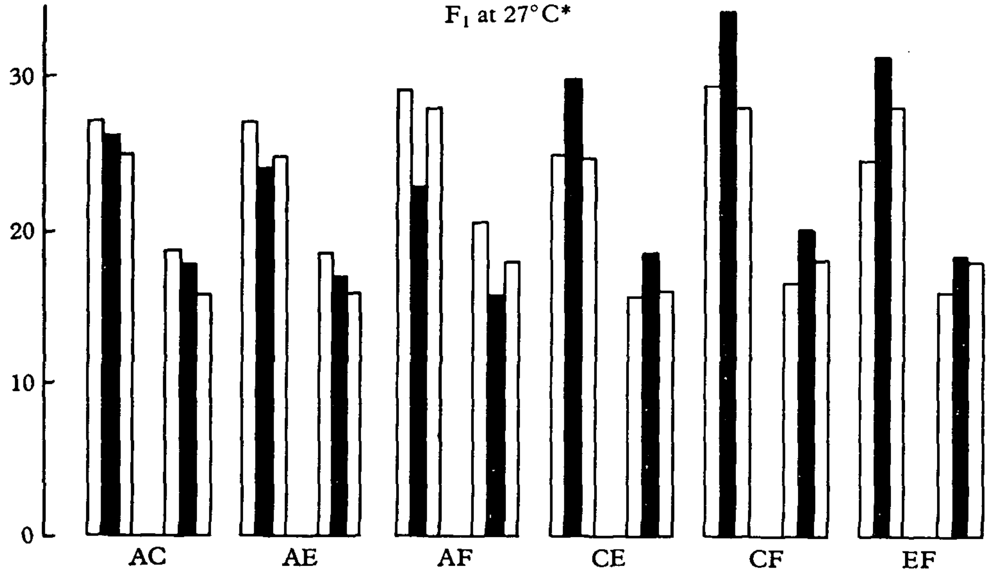
Pop.	1st Experiment, parents and F_1 ♀	1st Experiment, parents and F_1 ♂	2nd Experiment, parents and F_1 ♀	2nd Experiment, parents and F_1 ♂	1st Experiment, parents and F_2 ♀	1st Experiment, parents and F_2 ♂	2nd Experiment, parents and F_2 ♀	2nd Experiment, parents and F_2 ♂
A	25·2 ± 0·73	16·5 ± 0·32	29·4 ± 0·85	20·8 ± 0·56	29·5 ± 0·84	20·8 ± 0·38	27·3 ± 0·97	16·4 ± 0·38
C	22·3 ± 1·01	14·9 ± 0·38	29·7 ± 0·86	16·8 ± 0·38	30·9 ± 0·97	19·0 ± 0·34	27·8 ± 0·96	16·7 ± 0·61
E	21·4 ± 0·93	14·2 ± 0·38	28·3 ± 0·75	18·0 ± 0·53	28·8 ± 0·8	20·0 ± 0·42	27·3 ± 0·76	19·6 ± 0·40
F	— —	— —	28·2 ± 0·88	18·1 ± 0·44	26·7 ± 0·62	17·0 ± 0·34	27·4 ± 0·73	17·6 ± 0·33
AC	22·9 ± 0·78	16·1 ± 0·34	29·7 ± 0·80	19·4 ± 0·60	28·4 ± 0·83	20·3 ± 0·55	26·8 ± 0·8	19·3 ± 0·44
AE	21·5 ± 0·93	15·2 ± 0·35	26·9 ± 0·77	18·9 ± 0·47	— —	— —	— —	— —
AF	— —	— —	23·0 ± 1·2	15·9 ± 0·41	— —	— —	— —	— —
CE	27·1 ± 0·96	16·9 ± 0·40	32·8 ± 0·75	20·5 ± 0·50	27·4 ± 0·98	16·5 ± 0·40	25·8 ± 0·71	17·1 ± 0·34
CF	— —	— —	34·5 ± 0·77	20·3 ± 0·53	32·1 ± 0·95	21·1 ± 0·52	30·8 ± 0·85	18·4 ± 0·47
EF	30·2 ± 1·04	16·8 ± 0·47	32·7 ± 0·61	19·9 ± 0·57	24·1 ± 0·69	16·9 ± 0·38	22·8 ± 0·54	15·5 ± 0·32

Origin of populations: A maintained at 16°C.
C maintained at 25°C.
E and F maintained at 27°C.

Table 2. *Parents and hybrids at 16°C. Mean longevity (in days) of different populations and their hybrids*

	1st Experiment, parents and F_1		2nd Experiment, parents and F_1		1st Experiment, parents and F_2		2nd Experiment, parents and F_2	
Pop.	♀	♂	♀	♂	♀	♂	♀	♂
A	82·7 ± 2·54	39·3 ± 1·43	69·4 ± 1·92	38·6 ± 1·21	79·9 ± 1·79	38·0 ± 0·94	78·0 ± 1·78	37·7 ± 1·09
C	90·6 ± 2·85	41·6 ± 1·76	72·3 ± 2·52	46·8 ± 1·52	90·1 ± 2·92	45·1 ± 1·27	88·2 ± 2·28	45·9 ± 1·73
E	78·9 ± 3·07	33·3 ± 1·34	69·7 ± 1·74	41·0 ± 1·28	74·4 ± 2·41	37·2 ± 1·12	78·8 ± 2·19	31·6 ± 0·95
F	— —	— —	78·7 ± 1·17	40·1 ± 1·33	83·6 ± 2·21	37·9 ± 1·65	82·2 ± 1·94	34·0 ± 1·05
AC	87·8 ± 3·08	44·6 ± 1·84	74·0 ± 2·64	46·5 ± 1·4	76·8 ± 2·38	52·1 ± 1·77	82·6 ± 2·51	50·6 ± 1·21
AE	88·8 ± 3·50	35·0 ± 1·45	81·0 ± 2·28	44·5 ± 1·51	— —	— —	— —	— —
AF	— —	— —	75·1 ± 2·15	36·0 ± 1·18	— —	— —	— —	— —
CE	95·0 ± 3·12	40·8 ± 1·49	78·3 ± 2·39	48·1 ± 1·34	83·7 ± 2·68	37·9 ± 1·01	75·9 ± 2·61	34·0 ± 0·90
CF	— —	— —	80·1 ± 1·98	44·4 ± 1·52	100·7 ± 3·57	53·1 ± 1·76	91·0 ± 3·94	41·5 ± 1·79
EF	102·8 ± 3·55	32·2 ± 1·23	86·9 ± 2·25	41·7 ± 1·3	74·5 ± 1·66	41·7 ± 1·03	67·2 ± 1·68	35·1 ± 1·07

Origin of populations: A maintained at 16°C.
C maintained at 25°C.
E and F maintained at 27°C.



* Averages of 2 replicate experiments except for AF and CF

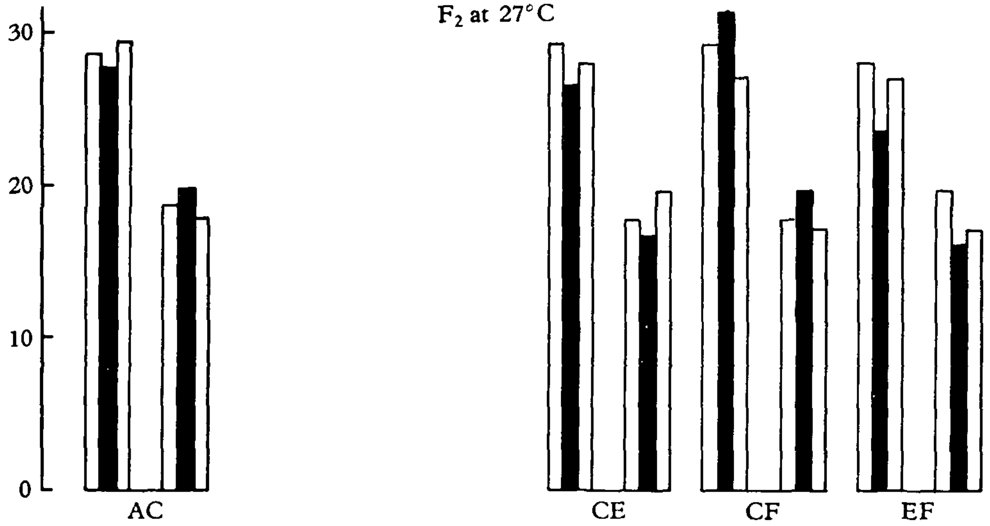


Fig. 1. Mean longevity, in days, of the parental populations and the F_1 hybrids (above), and of the parental populations and the F_2 hybrids (below), in experiments conducted at 27°C. In each comparison, the three bars to the left are females, those to the right are males.

Origin of populations: A maintained at 16°C.
C maintained at 25°C.
E and F maintained at 27°C.

population A as one of the parents. The only cross of this group examined in the F_2 was A × C. The F_1 and F_2 of this cross were not different from their respective mid-parent values. In other words, there was neither an F_1 superiority nor an F_2

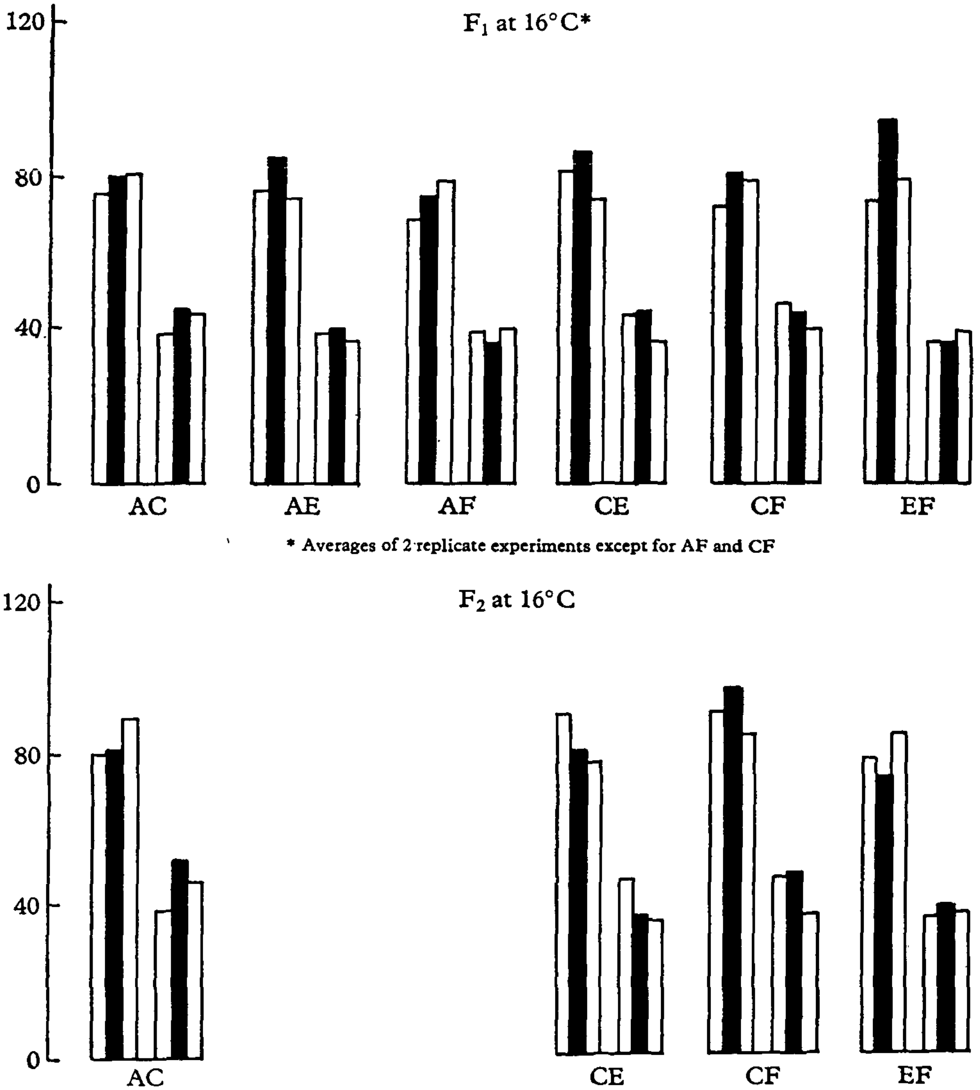


Fig. 2. Mean longevity, in days, of the parental populations and the F$_1$ hybrid (above), and of the parental populations and the F$_2$ hybrids (below), in experiments conducted at 16°C. In each comparison, the three bars to the left are females, those to the right are males.

Origin of populations: A maintained at 16°C.
C maintained as 25°C.
E and F maintained at 27°C.

breakdown. On the other hand, the F$_1$ from the crosses involving populations C, E, and F lived significantly longer than their respective parents. The F$_2$ hybrids from both C × E and E × F crosses showed clear evidence of the segregation effect, i.e., an F$_2$ breakdown. However, the F$_2$ from C × F did not show any evidence of breakdown, and in fact was significantly superior in longevity to both parents.

The results obtained show that the six populations did diverge genetically from each other in the course of the four and a half years when they were separated and some of them lived at different temperatures. It seems that a genetic divergence has taken place even between populations that were kept at the same temperature; this is shown by the results of crossing populations E and F. Populations A and C, which were kept at 16°C. and 25°C. respectively, lived at 27°C. as long as or even slightly longer than populations E and F which were kept at 27°C. all the time. Taking these results at their face value, it seems that there was no divergence of the populations in accordance with the temperatures of their environments.

Table 3. *Comparisons between the mid-parent values and the F_1 and F_2 values in the crosses between the populations (females only)*

	Tests at 27°C.			
	F_1—M.P.		F_2—M.P.	
Cross	I	II	I	II
A × C	− 0·9	+ 0·15	− 1·8	− 0·75
A × E	− 1·8	− 1·95*	—	—
A × F	—	− 5·80*	—	—
C × E	+ 5·2*	+ 3·80*	− 2·45*	− 1·75
C × F	—	+ 5·55*	+ 3·3*	+ 4·50*
E × F	+ 8·8*	+ 4·45*	− 3·65*	− 4·55*
	Tests at 16°C.			
A × C	+ 1·14	+ 3·15	− 8·2*	− 0·50*
A × E	+ 8·0*	+ 11·45*	—	—
A × F	—	+ 1·05	—	—
C × E	+ 10·20*	+ 7·30*	+ 1·45	− 7·6*
C × F	—	+ 4·60	+ 13·85*	+ 5·8
E × F	+ 23·9*	+ 12·70*	− 4·5	− 13·3

* Indicates significance at the 0·05 level of probability.

It may be that population A, and to some extent also population C, still retain a considerable amount of the genetic variability generated in the gene pool of their founders by the four-way hybridization. The selection pressure did not rigorously differentiate the gene arrays, since 16°C. is close to the optimum temperature, and 25°C. is not very far from it, for *D. pseudoobscura*. The fact that the F_1 hybrids resulting from crossing population A with other populations did not show significant differences from the mid-parent values favours this explanation. This does not explain, however, the persistence of increased longevity in the F_2 hybrids resulting from the cross C × F.

Populations E and F were exposed to a more stringent selection than populations A and C. The former populations were exposed to a sub-lethal temperature, 27°C., and to the effects of overcrowding in the population cage. Moreover, because of the higher temperature, they passed through at least twice as many generations as population A. All these factors acting together may have favoured the divergence of the gene pools in the three populations. The F_1 resulting from crossing populations E and F lived much longer in both replicate experiments than any of the parents, and the F_2 showed a pronounced breakdown in both cases.

In the experiments conducted at 16°C., the picture is somewhat different. Here again the F_1 and F_2 resulting from the cross A × C were intermediate between the parental populations. But the cross A × E yielded a superior F_1 in both replicate experiments, which was not the case at 27°C. This situation could be explained as a temperature-dependent effect. Unfortunately, the F_2 from this cross, A × E, was not examined to see whether this F_1 superiority would disappear in F_2. The other crosses involving population A, i.e., A × F, gave an F_1 which was not significantly different in longevity from the mid-parent value, whereas this same F_1 was significantly below the mid-parent value at 27°C. (Table 3). The crosses involving the populations C, E, and F yielded results that were almost identical with those obtained at 27°.

SUMMARY

Six experimental populations of *Drosophila pseudoobscura* were maintained in three different environments for almost four and a half years. All the populations have originally descended from the same founders. The populations were examined for evidences of genetic divergence. The longevity of the flies from four of these populations, and their F_1 and F_2 hybrids, was studied at 16°C. and at 27°C. The results indicate that the gene pools of some of the populations have diverged from each other.

REFERENCES

DOBZHANSKY, TH. & LEVENE, H. (1951). Development of heterosis through natural selection in experimental populations of *D. pseudoobscura*. *Amer. Nat.* **85**, 247–264.

EHRMAN, L. (1964). Genetic divergence in M. Vetukhiv experimental populations of *D. psuedoobscura*. I. Rudiments of sexual isolation. *Genet. Res.* **5**, 150–157.

MCFARQUHAR, A. M. & ROBERTSON, F. W. (1963). The lack of evidence for co-adaptation in crosses between geographical races of *D. subobscura*. *Genet. Res.* **4**, 104–131.

VETUKHIV, M. (1953). Viability of hybrids between local populations of *D. pseudoobscura*. *Proc. nat. Acad. Sci., Wash.* **39**, 30–34.

VETUKHIV, M. (1954). Heterosis and hybrid breakdown in hybrids between local populations of certain species of Drosophila. *Caryologia*, **6**, 760–761 (supplement).

VETUKHIV, M. (1956). The longevity of hybrids between local populations of *D. pseudoobscura*. *Acta genet.* **6**, 252–254.

VETUKHIV, M. (1957). Longevity of hybrids between geographic populations of *D. pseudoobscura*. *Evolution*, **11**, 348–360.