Genet. Res., Camb. (1966), 7, *pp.* 255–266
With 1 *text-figure*
Printed in Great Britain

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

3. DIVERGENCE IN BODY SIZE

By WYATT W. ANDERSON
The Rockefeller University, New York City

(*Received* 24 *July* 1965)

1. INTRODUCTION

Geographical gradients in body size have been described in several species of *Drosophila*. The genetic differences between populations of different localities, as revealed by differences in size among populations raised under the same conditions, generally parallel the phenotypic responses to the temperatures in which the *Drosophilae* develop. Flies from cooler regions tend to be genetically larger than flies from warmer regions; it is well known that *Drosophila* raised at lower temperatures are larger than those of the same strain raised at higher temperatures.

Stalker & Carson (1947, 1948) found in *Drosophila robusta* a trend to increased wing length with increasing latitude and with increasing altitude. Thorax length, like wing length an index of general body size, increased with altitude but not with latitude. In *D. subobscura*, Prevosti (1955) and Misra & Reeve (1964) found positive correlations between body dimensions and latitude, flies from cooler regions being larger. There is no evidence of a general cline in size correlated with latitude over the range of *D. pseudoobscura* (Sokoloff, 1965). As Sokoloff notes, however, the complex range of the territory which *D. pseudoobscura* inhabits may obscure such correlations. Ray (1960) raised four species of *Drosophila* (*willistoni*, *equinoxalis*, *pseudoobscura*, and *persimilis*), the sample of each species coming from a single locality, at temperatures varying between 16°C. and 29°C. For each species there were striking increases in size, measured as either wet body weight or as wing length, at lower as compared to higher temperatures. For a 10°C. temperature interval there was an average difference of 30·5% in wet weight and 17·3% in wing length.

The experimental populations begun by Dr M. Vetukhiv offer an opportunity to study the selective effects of temperature on populations of *D. pseudoobscura*. These populations, genetically identical at the beginning, have been maintained at three different temperatures for over 7 years. The purpose of the present work is to determine whether the relationships of body size to environmental temperature found in some natural populations of *Drosophila* might be paralleled in Vetukhiv's experimental populations.

2. MATERIALS AND METHODS

(i) *The experimental populations*

In May 1958 Dr Vetukhiv established six experimental populations of *Drosophila pseudoobscura*, all derived from the same group of about 1000 founders. The founders were the double-cross progenies of about forty strains of *D. pseudoobscura* collected in four localities in California, Utah, and Colorado. All the founders were monomorphic for the Arrowhead gene arrangement in the third chromosome, thus excluding any possible complications from inversion polymorphism. The populations have been kept at three different temperatures. Populations A and B have been maintained at 16°C., populations C and D at 25°C., and populations E and F at 27°C. The population cages used have been described by Wright & Dobzhansky (1946); these cages support large populations, varying from about 1000 to about 4000 individuals. For further details on Vetukhiv's populations, see Ehrman (1964).

(ii) *Measurements*

The length of the wing along the third longitudinal vein from the outer margin of the anterior crossvein to the tip of the wing has been used as a measure of body size. Left wings were removed and mounted in Canada balsam for later measurement. The measurements were made under a compound microscope at magnification × 63, with an ocular micrometer of 100 divisions. At 19°C. the average female wing measured about 90 scale divisions, and the average male wing 80 divisions. Wing length was recorded to the nearest unit of the micrometer scale. A unit on the ocular micrometer scale corresponds to 20·8 μ.

For the determination of wet body weight, small groups, containing nine flies on the average, were weighed on a chemical balance registering to 0·1 mg. Males and females were weighed separately when they were 6 to 9 hours old. The average female weighed 1·25 mg. at 25°C., and the average male 0·98 mg.

To measure the developmental time, eggs were collected over an 8–12 hour period, and samples of 50 eggs were placed in each of eleven replicate bottles for each population. The number of adults appearing was recorded once each day. Since almost all flies hatch early in the morning, counting was done late in the afternoon to insure fully expanded wings for the determination of wing length.

(iii) *Design of the experiments*

The following procedure was adopted for all the experiments reported in this paper. Samples of approximately 1000 eggs were taken from each cage, subdivided among six bottles, and incubated at either 19°C. or 25°C. The adults coming from the initial egg sample were then placed in vials with spoons containing Kalmus' (1943) medium, blackened with charcoal, for the collection of eggs.

Several hundred parents were used per population, distributed over five to ten vials. Counted samples of either 50 or 100 eggs were then placed in yeasted half-pint bottles with Spassky's (1943) Cream-of-Wheat medium. For each experiment these bottles were kept at the same temperature in which the initial egg sample was incubated. (The only exception to this routine was the study of wing length at 16°C.; in this one study the initial egg sample was incubated at 25°C. and the measurements made on flies raised at 16°C.) The adults emerging in these bottles were measured. Thus, all of the flies actually measured were one generation removed from their cages and temperatures of origin and were raised under uncrowded, nearly optimal conditions. This procedure should have eliminated possible effects on the eggs of the different environmental temperatures at which Vetukhiv's populations were maintained. In all experiments wings were removed from a random sample of all the flies hatching in a given culture. All experimental cultures were kept in circulating-air incubators in which the temperatures only rarely varied as much as 0·5°C. on either side of the desired temperatures. Bottles were randomized and, wherever possible, all the bottles for a single experiment were kept on the same shelf within the incubator.

The first experiments, those on wet body weight at 25°C. and wing length at 16°C., had 100 eggs in each of four replicate bottles per cage. All flies emerging were measured. On analyzing the data from these two experiments it became apparent that the variance between replicate bottles was large compared to the variance within bottles, undoubtedly a reflexion of the unavoidable variations in food, humidity, and yeast among the culture bottles. Accordingly, all the later experiments were set up with eleven replicate bottles, each containing 50 eggs, for each population studied. Ten wings per sex (where both sexes were studied) from each of ten bottles were measured per population. In many experiments only female wings were measured. A separate set of parental cultures were raised simultaneously with both F_1 and F_2 hybrid generations. Reciprocal crosses were made for each combination of parental cages; ten female wings from each of five replicate bottles were measured for each parental population and for each reciprocal of the hybrid crosses. The comparisons F_1-midparent and F_1-F_2 were thus based on comparisons between sets of ten replicate cultures.

(iv) *Repeatability*

The two experiments carried out at 19°C. involving the parental populations give an idea of the repeatability of body size measurements. The first set of mean wing lengths is the average of the parental populations used for comparisons with the F_1 and F_2 hybrids (19°C. I in Fig. 1). The second set (19°C. II in Fig. 1), obtained 8 months later, agrees well with the first.

(v) *Statistical techniques*

Within each sex at each temperature, there was no evidence of a dependence of within-bottle variance on the mean body size. The statistical analyses were

therefore carried out on the untransformed data. Separate analyses were made for each sex at each temperature.

Error variances are based on variance within culture bottles and on variance between replicate bottles. The between-bottle mean square was significantly greater ($P < 0{\cdot}01$) than the within-bottle mean square for every experiment. This reflects greater environmental differences between bottles as compared to the relatively uniform environments within individual bottles. All but the earliest experiments were designed to minimize the error variance by increasing the number of replicates for each population being studied.

The significance of comparisons planned at the outset of the experiments was judged by *t*-tests. In this category were the comparisons of mean body size in 'cold' populations (A and B) with mean body size in 'warm' populations (C, D, E ,and F), the comparisons of reciprocal crosses in the F_1 and F_2 hybrids, and the comparisons of F_1 and midparent and F_1 and F_2. Unplanned comparisons between mean body sizes of all pairs of parental populations were made with Scheffe's test. Standard errors of the population means were obtained from pooled error variances. The variability of parental populations and the F_1 and F_2 hybrid crosses between them were compared as ratios of pooled within-bottle variances.

3. RESULTS

(i) *Phenotypic modification of body size by temperature*

Since the aim of the present study was to explore the possible selective effects of temperature on body size, the phenotypic effects of temperature on body size may usefully be described first. Several hundred adult flies were taken from a population cage descended from flies collected at Berkeley, California. They were allowed to oviposit on spoons with Kalmus' medium at 25°C.; samples of 50 eggs were then placed in each of thirty bottles with the Cream-of-Wheat medium; these were equally divided among incubators kept at 19°, 25°, and 27°C. Ten females were taken at random from the progeny and their left wings measured. The mean sizes ± their standard errors were (1 unit = 20·8 μ):

19°C.	25°C.	27°C.
87·58 ± 0·57	79·01 ± 0·23	74·81 ± 0·33

The average difference between the flies which developed at 19°C. and 25°C. is about 11% of the wing length at 25°C., while that between 25°C. and 27°C. is only about 5% of the same value.

In another experiment, eggs were obtained from flies which developed in each of the six Vetukhiv's populations. Samples of 100 eggs were placed in each of eight bottles, four of which then developed at 16°C. and four at 25°C. The wings of approximately 200 females per population per temperature were measured. All populations showed approximately the same response; the pooled data are as follows:

16°C.	25°C.
92·32 ± 0·57	79·03 ± 0·46

Table 1. *Mean body weight, wing length, and developmental time*

		Populations						
		A	B	C	D	E	F	S.E.*
A. Experiments at 1½ years								
Wing length[1]	♀ at 15°C.	88·35	88·45	88·85	90·95	89·70	89·25	0·80
	♂ at 15°C.	81·90	81·95	82·05	83·00	82·20	82·20	0·65
	♀ at 25°C.	78·75	78·70	79·00	79·95	79·90	78·95	0·70
	♂ at 25°C.	73·05	72·85	73·35	74·30	74·00	74·00	0·60
B. Experiments at about 6 years								
Body weight (mg.)	♀ at 25°C.	1·26	1·33	1·18	1·18	1·24	1·16	0·06
	♂ at 25°C.	1·01	1·08	0·95	0·94	0·98	0·92	0·04
Wing length[1]	♀ at 16°C.	93·47	94·55	92·44	91·60	91·47	90·72	0·57
	♀ at 19°C.	91·54	92·57	89·40	87·82	89·15	87·46	0·40
	♂ at 19°C.	84·22	84·52	81·80	80·10	82·11	80·34	0·34
	♀ at 25°C.	82·11	83·36	79·78	79·79	80·88	76·86	0·35
Developmental time (days)	♀ at 19°C.	19·83	19·86	20·12	20·05	19·72	20·03	0·09
	♂ at 19°C.	20·66	20·66	20·01	20·92	20·57	20·86	0·11

* Standard error for every mean in a given experiment, obtained from pooled error variance.
[1] One unit = 20·8 μ.

Table 2. *Analysis of variance for body size*

			Females			Males		
			df	MS	F	df	MS	F
A. Experiment at 1½ years								
Wing length	at 15°C.	Cages	5	94·25	1·47	5	16·25	—
		Error	24	64·25		24	41·50	
	at 25°C.	Cages	5	32·25	—	5	33·75	—
		Error	24	51·50		24	37·50	
B. Experiments at about 6 years								
Wing length	at 16°C.	Cages	5	408·94	6·22**	—	—	—
		Error	17	65·75		—	—	
	at 19°C.	Cages	5	410·76	26·18**	5	349·64	29·76**
		Error	54	15·69		54	11·75	
	at 25°C.	Cages	5	478·06	39·32**	—	—	—
		Error	55	12·16		—	—	
Body weight (mg.)	at 25°C.	Cages	5	0·64	1·29	5	0·51	2·10
		Error	18	0·49		18	0·25	
Developmental time (days)	at 19°C.	Cages	5	5·95	2·71*	5	8·12	2·41*
		Error	60	2·20		60	3·37	

* and ** indicate significance at 0·05 and 0·005 levels.
[1] One unit = 20·8 μ.

https://doi.org/10.1017/S0016672300009666 Published online by Cambridge University Press

The size difference between the flies raised at 16°C. and at 25°C. is 17% of the wing length at 25°C., which is, as expected, greater than that observed above for flies raised at 19°C. and 25°C.

(ii) *Lack of early divergence in body size*

When Vetukhiv's populations were about 1½ years old, Mrs M. Krimbas measured their wing lengths. Adults taken from each population were allowed to oviposit and

Table 3. *Comparison of mean body sizes in 'cold' populations (A and B) and in 'warm' populations (C, D, E, and F)*

		½(A+B)−½(C+D+E+F)	
		♀	♂
A. Experiment at 1½ years			
Wing length[1]	at 15°	−1·30	−0·45
	at 25°	−0·70	−0·95
B. Experiments at about 6 years			
Body weight (mg.)	at 25°	0·10*	0·10*
Wing length[1]	at 16°	2·45**	—
	at 19°	3·60**	3·28**
	at 25°	3·41**	—
Developmental time (days)	at 19°	−0·13*	−0·18*

* and ** indicate significance at 0·05 and 0·001 levels.
[1] One unit = 20·8 μ.

the eggs were placed in equal numbers in ten replicate bottles. Five bottles were raised at 15°C. and the other five at 25°C. Twenty females and twenty males were measured from each bottle. In no case was a significant difference observed (Tables 1, 2, and 3). Mrs Krimbas recorded wing length on a micrometer scale with units of 104 μ.

(iii) *Body size in the populations at about 6 years of age*

Between the spring of 1963 and the spring of 1965, when the populations were between 5 and 7 years old, measurements of wet body weight and of wing length were made at temperatures of 16°, 19° and 25°C. Table 1 gives the mean values and their standard errors for each population, and Table 2 the analysis of variance for these experiments. The standard errors for all populations in any particular experiment are based on the pooled error variance for that experiment. The differences in wing length among the populations are statistically highly significant. For wet body weight the differences are consistent with the results of the wing measurements, but the variance between replicate bottles was large enough to obscure such differences as apparently did exist. In all experiments, the means show

a clear pattern, illustrated in Fig. 1. At all temperatures there is a difference between the two 'cold' populations maintained at 16°C., and the four 'warm' populations maintained at 25°C. and 27°C. In Table 3 the mean body size in the 'cold' populations is compared with mean body size of the 'warm' populations. The populations from 25°C. and 27°C. have been lumped together in this comparison, since the mean body size in the two populations from 25°C. is nearly identical with

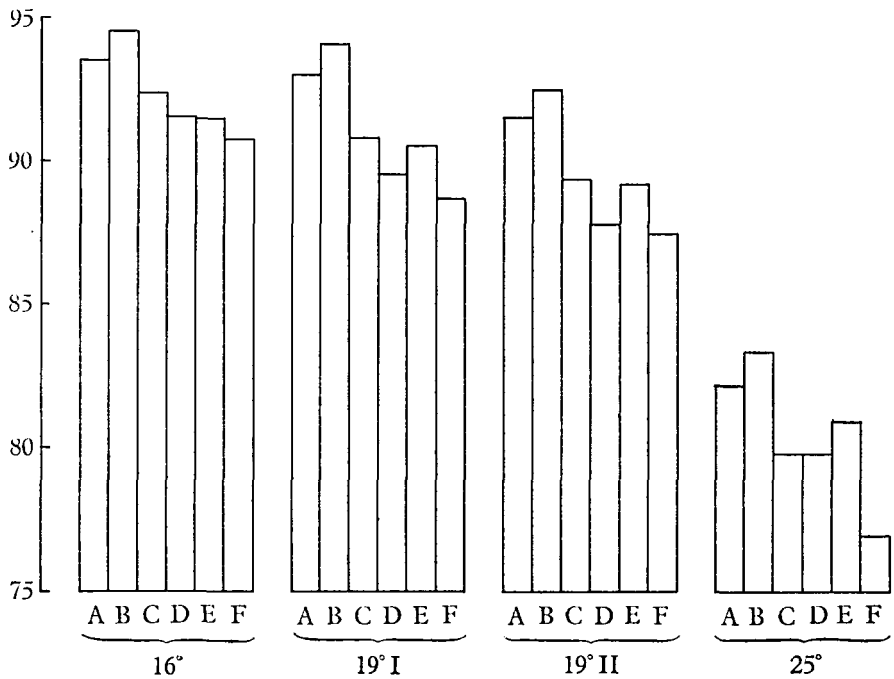

Fig. 1. Wing length of females from Vetukhiv's populations in tests at three temperatures. Note that ordinate begins at 75 units. Length in units of micrometer scale, 1 unit = 20·8 μ.

the mean body size in the two populations from 27°C. in most of the experiments presented here. Actually, body size in population E from 27°C. was quite similar, sometimes slightly larger, than body size in populations C and D from 25°C. Body size in population F from 27°C. was consistently smaller than in any of the other populations.

An idea of how striking is the divergence among Vetukhiv's populations can be gained from comparisons between all pairs of means. These comparisons are given in Table 4 for wing length of females and males at 19°C. and for wing length of females at 25°C. The difference between the largest mean size, always in population B from 16°C., and the smallest mean size, always in population F from 27°C., was 8% of the average body size in the experiment at 25°C. and 6% of the average body size in the experiment at 19°C.

Unfortunately, these experiments do not allow a comparison of absolute sizes of the flies in the experiments at $1\frac{1}{2}$ and at 6 years. Differences in food medium

cooked at different times and in other environmental conditions change the absolute body sizes. For instance, the average size of the parental flies raised with the F_2 hybrids was 2% larger than that of the parental flies raised with the F_1 hybrids.

Table 4. *Statistical significance of differences in mean wing length at 6 years (ns = not significant)*

A. Experiment at 19°C. ♀'s above diagonal, ♂'s below

	A	B	C	D	E	F
A		ns	0·05	0·005	0·01	0·005
B	ns		0·005	0·005	0·005	0·005
C	0·005	0·005		ns	ns	0·05
D	0·005	0·005	0·05		ns	ns
E	0·005	0·005	ns	0·01		ns
F	0·005	0·005	ns	ns	0·05	

B. Experiment at 25°C. ♀'s only

	A	B	C	D	E	F
A		ns	0·005	0·005	ns	0·005
B			0·005	0·005	0·005	0·005
C				ns	ns	0·005
D					ns	0·005
E						0·005
F						

(iv) *Time of development*

The time of development from egg to adult and wing length were measured in the same flies in an experiment at 19°C. The time of development is significantly different among the six populations (Table 2). The mean time of development (Table 1) is consistently shorter in the populations with larger flies. Mean time of development in the progenies of the flies derived from the two 'cold' cages was significantly shorter than the mean for the four 'warm' cages (Table 3).

(v) *Studies of F_1 and F_2 hybrids*

With two exceptions, the reciprocal crosses for each F_1 and F_2 hybrids were not significantly different. The exceptions involved population C. The difference between the crosses C♀ × A♂ and A♀ × C♂ was statistically highly significant ($P < 0{\cdot}001$), CA being the larger. This effect persisted into the F_2 generation, the F_2 derived from the C♀ × A♂ F_1 being larger than the F_2 derived from the A♀ × C♂ F_1. The crosses C♀ × F♂ and F♀ × C♂ also suggested a possible maternal effect on size in population C. Unfortunately, all but two bottles of crosses FC (and FA) were lost; although the two remaining bottles contained sufficient flies to obtain an F_2 generation in each case, no accurate data can be given for the F_1 generation. A statistically highly significant ($P < 0{\cdot}01$) difference between the F_2's of crosses

C♀ × F♂ and F♀ × C♂ was found, CF being the larger. Thus, a maternal effect on body size possibly exists in population C. But in the absence of data from all relevant crosses, and in the absence of measurements on males, our judgement on the reality of this effect must be reserved.

The two hybrid generations were raised simultaneously with a parental generation. The parental samples raised with the F_2 hybrids were about 2% larger on the average than the parental samples raised with the F_1's. The F_2 means were adjusted

Table 5. *Comparison of wing lengths in hybrids and parents; females only, at* 19°*C.*

Cross[1]	F_1-midparent†	F_1-F_2†
AB and BA	−0·26	−0·35
AC	−0·71	0·78
CA	1·65**	−0·49
AE and EA	1·26**	1·00*
AF and FA	−0·05	1·08*
BE and EB	1·15**	0·39
BF and FB	0·85*	0·23
CF	2·02***	−0·30
EF and FE	1·43***	0·47

*, **, and *** indicate significance at 0·05, 0·01, and 0·001 levels respectively.
[1] Female parent given first; i.e., AB = A♀ × B♂.
† One unit = 20·8 μ.

by this factor before comparison with the F_1 means. The comparisons F_1-midparent are given in Table 5; in six of the nine comparisons the difference is significant, the F_1 hybrid being larger than the mean of its parents. The comparisons of F_1-F_2 are given in Table 5; in two of the nine comparisons the F_2 was significantly smaller than the F_1. The pooled within-bottle variances of the parental populations and of the F_1 and F_2 hybrids between them are compared in Table 6. There is no evidence of a difference in variability between either the F_1 or F_2 generations and the parental populations raised simultaneously with each.

Table 6. *Comparison of pooled within-bottle variances of wing length in parents and hybrids; females only, at* 19°*C.*

	df	ss	ms	F
(a) Parents	270	732·9	2·71	1·01
F_1	621	1667·4	2·69	
(b) Parents	270	975·3	3·61	1·15
F_2	720	2256·6	3·13	

4. DISCUSSION

Vetukhiv's populations were initially genetically identical but heterogeneous. The differences in body size among the populations kept at different temperatures were at first only phenotypic. Flies in the two populations at 16°C. were about 17%

larger, as measured by wing length, than flies in the two populations at 25°C. Flies in the two populations at 27°C. were, however, only a little smaller, 5% on the average, than the flies at 25°C. Thus, the phenotypic differences among Vetukhiv's populations in their early stages were chiefly those between the two populations at 16°C. and the four populations at 25°C. and 27°C.

After 1½ years there was no indication that the populations were diverging genetically in body size. The changes in body size observed later clearly did not result from a rapid selection in the early generations. The experiments at 5 to 7 years disclosed that a striking divergence among the populations had occurred. The size differences once induced by different environmental temperatures alone have now become in part genetically assimilated. For both wet body weight and for wing length, the flies in the populations kept at 16°C. are genetically determined for larger size than the flies kept in the populations at the higher temperature, 25°C. and 27°C. In the populations kept at different temperatures the exact pattern varies somewhat; there are sometimes significant differences between replicate populations kept at the same temperature. But over and above these variations there is a clear distinction between those populations kept at lower and those kept at higher temperatures.

The divergence in body size among Vetukhiv's populations is impressive. The genetic difference between the mean sizes in the population with the largest and in that with the smallest flies is, at the same temperature, over half the total phenotypic change between the two extreme temperatures at which the populations have been maintained, 16°C. and 27°C. Chance occurrence of such changes is extremely unlikely. The populations have been too large for random genetic drift to have an appreciable effect, and the results of the study at 1½ years rule out the possibility of a rapid reorganization of gene pools during the initial adaptation of the flies to the different environments afforded by the six population cages. That the changes show as clear a pattern as they do, suggests that selection has favored larger body size at the lower temperature and smaller body size at the higher temperatures. This selection has acted slowly to produce a gradual genetic divergence of the populations. The differences in body size are accompanied by differences in the time of development from egg to adult, the faster developers being the larger flies. The target character for the selection may not be body size itself, but some other character which is genetically highly correlated with body size. In this case the effect of the selection, whether in the laboratory or in nature, will still be to produce genetic differences in body size. The changes observed in Vetukhiv's populations are examples of the selective process Waddington (1953, 1961) has called 'genetic assimilation'.

Ehrman (1964) and Mourad (1965) found that Vetukhiv's populations had diverged also with respect to mating behavior and longevity. There is no pattern with temperature, however, for the differences in longevity and mating behavior. The genetic nature of the differences in body size among Vetukhiv's populations can to some extent be inferred from the hybrid studies. There is a partial dominance, or heterosis, of larger size, the F_1's being significantly larger than the parental mean

in most crosses. This heterosis is not accompanied by changes in the variability of the hybrid generations. In two of the nine crosses, the F_2 flies are significantly smaller than their F_1 parents.

It is of interest to compare our results with those of Druger (1962), who subjected *Drosophila pseudoobscura* to selection for body size; the selection was practiced in lines kept at different temperatures, and then the selected lines were tested also at other temperatures. The results of selection for body size at low and at high temperatures were qualitatively similar when the selected lines were compared over a broad range of temperatures. The precise quantitative relationships, however, did depend on the temperatures at which the lines were selected and the temperatures at which the selected lines were compared. Vetukhiv's populations, which have undergone natural selection for body size, show a behavior similar to Druger's artificially selected lines. Qualitatively, the distinction between the populations from 16°C. and the populations from 25°C. and 27°C. is clearly revealed at all temperatures of comparison—16°, 19°, and 25°C. But the exact pattern of sizes varies according to the temperature at which the populations are compared.

The changes in body size in Vetukhiv's cages of *D. pseudoobscura* may also be compared with those found by McFarquhar & Robertson (1963) among geographic races of *D. subobscura*. Like Vetukhiv's populations, the populations of *D. subobscura* were genetically heterogeneous, differing widely in body size. But there was no evidence of a departure from additivity in the crossses between the populations of *D. subobscura*, although they differed by as much as 20% in size. As mentioned above, Vetukhiv's populations showed a pronounced nonadditivity in F_1 hybrids.

The temperature-directed selection for body size found in Vetukhiv's populations may well be similar to that which has produced the temperature-oriented gradients of body size in some natural populations of *Drosophila*. In nature, of course, many other selective factors affecting body size may complicate and even obscure the formation of clear gradients.

SUMMARY

1. Six initially identical populations of *Drosophila pseudoobscura* have been maintained in population cages for 7 years. Two populations have been kept at 16°C., two at 25°C., and two at 27°C.

2. One and a half years after the start, there was no significant genetic divergence in body size among the populations. When the populations were about 6 years old, a striking genetic divergence in body size was found. The genetic difference between the populations having the smallest and the largest mean sizes is over half the total phenotypic change in size between the two extreme temperatures at which the populations were kept. The populations kept at the lower temperature have genetically larger flies than the populations kept at the higher temperatures.

3. Accompanying the changes in body size were changes in the time of development from egg to adult, the faster developers being the larger flies.

4. The F_1 hybrids from crossses between Vetukhiv's populations showed non-additivity of the genes for body size, the F_1's in most cases being significantly larger than the midparent. There was no change in variability of body size in the F_1 or F_2 hybrids.

5. The temperature-directed selection for body size found in Vetukhiv's experimental populations may well be similar in kind to that which has produced temperature-oriented geographic gradients for body size in natural populations of several species of *Drosophila*.

It is a pleasure to acknowledge the advice and encouragement of Professor Th. Dobzhansky throughout the course of this work. I am grateful to Mrs M. Krimbas for the early data on wing length, and to Mr Boris Spassky, Mrs Olga Pavlovsky and Miss Pat Hall for many kindnesses during the course of this work. Mr Christopher Wills kindly furnished the population of *Drosophila pseudoobscura* from Berkeley.

REFERENCES

DRUGER, M. (1962). Selection and body size in *Drosophila pseudoobscura* at different temperatures. *Genetics*, **47**, 209–222.

EHRMAN, L. (1964). Genetic divergence in M. Vetukhiv's experimental populations of *Drosophila pseudoobscura*. 1. Rudiments of sexual isolation. *Genet. Res.* **5**, 150–157.

KALMUS, H. (1943). A factorial experiment on the mineral requirements of *Drosophila* culture. *Am. Nat.* **77**, 376–380.

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

MISRA, R. K. & REEVE, E. C. R. (1964). Clines in body dimensions in populations of *Drosophila subobscura*. *Genet. Res.* **5**, 240–256.

MOURAD, A. (1965). Genetic divergence in M. Vetukhiv's experimental populations of *Drosophila pseudoobscura*. 2. Longevity. *Genet. Res.* **6**, 139–146.

PREVOSTI, A. (1955). Geographical variability in quantitative traits in populations of *Drosophila subobscura*. *Cold Spring Harb. Symp. quant. Biol.* **20**, 294–299.

RAY, C. (1960). The application of Bergmann's and Allen's rules to the poikilotherms. *J. Morph.* **106**, 85–108.

SOKOLOFF, A. (1965). Geographic variation of quantitative characters in populations of *Drosophila pseudoobscura*. *Evolution, Lancaster, Pa.* **19**, 300–310.

SPASSKY, B. (1943). Cream of wheat-molasses fly medium. *Drosoph. Inf. Serv.* **17**, 67–68.

STALKER, H. & CARSON, H. (1947). Morphological variation in natural populations of *Drosophila robusta Sturtevant*. *Evolution, Lancaster, Pa.* **1**, 237–248.

STALKER, H. & CARSON, H. (1948). An altitudinal transect of *Drosophila robusta Sturtevant*. *Evolution, Lancaster, Pa.* **2**, 295–305.

WADDINGTON, C. H. (1953). Genetic assimilation of an acquired character. *Evolution, Lancaster, Pa.* **7**, 118–126.

WADDINGTON, C. H. (1961). Genetic assimilation. *Adv. Genet.* **10**, 257–293.

WRIGHT, S. & DOBZHANSKY, TH. (1946). Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. *Genetics*, **31**, 125–156.