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The lack of evidence for co-adaptation in crosses between geographical races of *Drosophila subobscura* Coll.

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1. INTRODUCTION

It is widely accepted that the gene arrays in highly heterozygous populations are mutually adjusted to minimize possible disadvantageous effects of segregation on fitness, and this is generally referred to as balance or co-adaptation. Disturbance of the genetic equilibrium in one way or another, e.g. by artificial selection, inbreeding or by introducing chromosomes from other different gene arrays, may be expected and has often been shown, to lower fitness. More detailed analysis of the genetic behaviour generally indicates non-allelic interaction, as in the extensive studies of the effects of inserting chromosome arrangements of *D. pseudoobscura* in different genetic backgrounds (Dobzhansky, 1949, 1954) in the analysis of the genetic effects of inbreeding and its dissipation (Robertson & Reeve, 1954; Breese & Mather, 1960) and by different kinds of selection (Robertson, 1954, 1959, 1962*b*).

Vetukhiv (1953, 1954, 1956, 1957) reported that crosses between strains derived from different localities frequently indicate differences in co-adaptation in a more vigorous or fertile F_1 and less fit F_2. At present, little or nothing is known about the spatial distribution in nature of differences in co-adaptation which can be detected in this way. Such differences would presumably be related to the degree of reproductive isolation, and average effective population size and differences in habitat between populations.

This general problem can be best elucidated by comparing different species, preferably those which differ sharply in breeding structure or kind of habitat. Breeding structure will be governed chiefly by average population size. Some species may exist as large stable populations, while others may lead a more precarious existence with recurring low effective size of population. In practice, for the genus *Drosophila* we have little precise information about the breeding structure of different species, and generally little information about the habitat of the larval stage either. Trapping of adults is the best guide at present, although it requires cautious interpretation since closely related species flying in the same wood may differ sharply in their response to the same bait, so that the relative frequency in traps may reflect olfactory differences rather than relative abundance. Intraspecific differentiation into co-adapted gene arrays can be regarded as the first step in speciation. The descendants of immigrants from sufficiently differentiated

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populations will be handicapped in comparison with non-immigrant individuals. As Haldane (1959) has implied, this is relevant to the likelihood that immigrants from populations at higher adaptive peaks (Wright, 1931) will upgrade the populations with less favourable gene arrays. The more precise the genetic integration within populations the less important is this likely to be.

The present paper describes a search for evidence of differences in co-adaptation in relation to geographical separation between populations of *Drosophila subobscura* by comparing the performance of different strains with that of the F_1 and the F_2 from crosses between them. This species is abundant and widely distributed in the temperate zone of Europe and Asia and extends into the Middle East. It can be trapped easily so that wild populations can be adequately sampled and it can be cultured satisfactorily in the laboratory, unlike many species of the genus. As in many other species, other than *D. melanogaster* and *D. funebris*, virtually nothing is known about its breeding habits in the wild—a perennial challenge which no one has successfully taken up yet. Therefore, we have no exact information about population structure, but have the impression that breeding sites are frequent in wooded habitats. On any criteria this seems to be as good a species with which to initiate a systematic survey as any other, but the value of data derived from this species will be greatly enhanced when we can compare it with evidence from other species known to differ in population structure. As we shall see, however, the results did not turn out quite as might have been expected.

2. MATERIAL AND METHODS

(i) *Trapping wild flies*

Samples have been derived from a number of British sites including an east–west transect across Scotland with trapping sites approximately every ten miles; in addition samples were trapped at several European sites located in Denmark, Switzerland and at the southern margin of the range in Israel. Using the methods recommended by Basden (1951), traps with a yeasted fruit bait were located as near deciduous trees as possible. When brought into the laboratory single wild females were put in vials, generally with one male, and so it was possible to identify fertile females. No population was started with less than thirty such females and usually the number was higher. Thereafter, at least six culture bottles were kept per generation and the flies hatching from these were randomized among bottles of the next generation to minimize any tendency to inbreeding.

(ii) *Criteria of performance*

Strictly speaking, differences in co-adaption should be assessed in terms of fitness with respect to the normal environment. This council of perfection cannot be followed for obvious reasons and we did not attempt to do so. Instead, several characters were studied, whose behaviour can be reasonably related to fitness, under several environmental conditions—such as chemically defined sub-optimal diets which provide a convenient way of exposing relevant differences which would

be concealed in more favourable environmental conditions. The evidence for or against differences in genetic integration between populations is based on the behaviour of these characters under such different environmental conditions.

The characters are body-size, the length of the period of development and survival, measured as the percentage of eggs which give rise to adults. Within-culture variance for body-size and development time has also been examined to see whether there was any evidence of increased effects of segregation on the phenotype. Body-size refers to the length of the thorax and all data have been converted to three times the natural log of this dimension in $\frac{1}{100}$ mm, so that differences on this scale are roughly equivalent to proportional differences in body-weight. Females only were scored.

Many tests have shown (Sang & Clayton, 1957; Clarke, Maynard Smith & Somdhi, 1961; Robertson, 1960*a*, and in press) that a lengthening of the larval period is a sensitive indicator of unfavourable changes in both genotype and environment. We might assume, therefore, that the speed of larval growth, especially under the stress conditions provided by sub-optimal diets, will reflect levels of co-adaptation which involve complex non-allelic interaction. The duration of development has been expressed as log days and includes the larval and pupal period. Since there is usually a considerable spread in the hatching time of adults, cultures were examined only once a day.

For analysis of the survival data an angular transformation was used for the percentage of eggs which gave rise to adults.

(iii) *Culture methods*

For culture on live yeast media the usual maize meal molasses medium was used after trying several alternatives. For sub-optimal diets larvae were grown on synthetic media which involved various modifications of Sang's synthetic medium C (1956). Although this was worked out originally for *D. melanogaster* it provides a suitable basis for preparing alternative diets for *subobscura* as well, although as will be shown later, this species differs from *melanogaster* in some respects. The principle sub-optimal diets were provided by restricting the level of protein, RNA or choline in the medium. General procedure for aseptic culture was the same as described elsewhere (Robertson, 1960*a*). Generally, four to five replicated cultures per genotype and treatment were set up and eight to ten females measured per tube for body size. All females were scored for development time and all flies hatching provided the measure of survival.

D. subobscura is adapted to a lower temperature range than *melanogaster*, so that culture at 25° reduces the survival and induces male sterility. Since the populations were drawn from localities with rather different average temperatures and since Prevosti (1955) has shown that there is a cline relating wing length and temperature during the larval period, experiments have been carried out at 18°, 22° and 25° to see whether gene-environment interaction with respect to temperature was of any importance for measures of co-adaptation. Estimates of error variance are based on the within- and between-culture effects, pooled over comparable series.

3. EXPERIMENTAL RESEARCH

(i) *A comparison of Scottish populations*

The wild flies were collected at 10-mile intervals in a line across southern Scotland numbered as shown in the map in Fig. 1. All possible crosses were made between flies from alternate sites (i.e. 2, 4, 6, 8 and 10) and were taken to an F_2. The test was

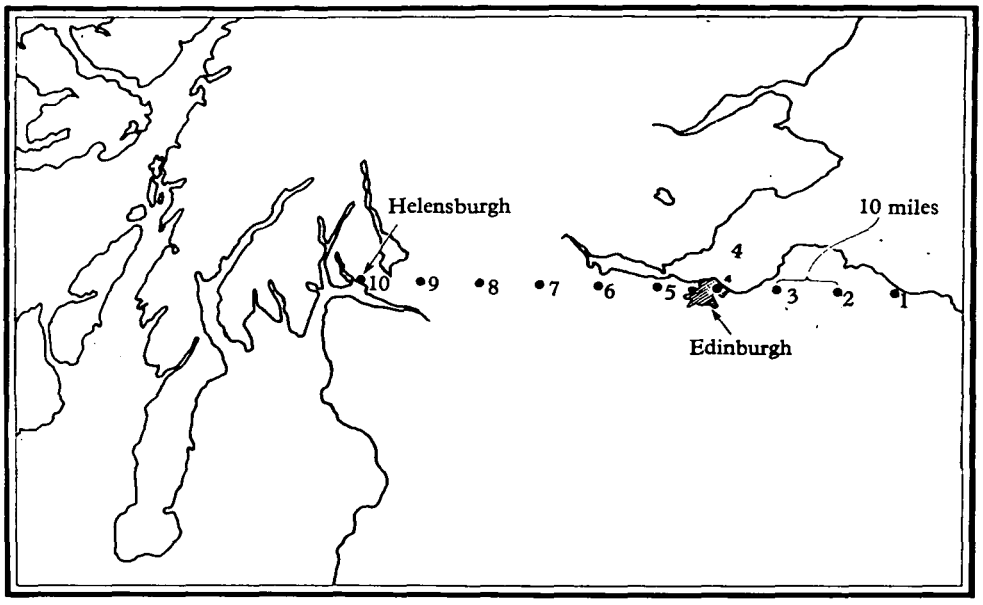

Fig. 1. The distribution of trapping sites in Scotland.

so arranged that samples from the parental populations, the 10 F_1's and the F_2's were raised at the same time on the standard aseptic medium at 18° C. Cultures of flies from sites 7, 9 and 10 and one F_2 series (8 × 10) failed.

Table 1. *Average body-size, development time and survival of flies from different Scottish sites*

Site	Body-size (logs)	Development time (logs)	Survival (%)
1	14·67	3·06	64·5
2	14·70	3·09	75·0
3	14·70	3·07	89·0
4	14·69	3·09	72·0
5	14·66	3·08	66·0
6	14·72	3·05	84·0
8	14·72	3·09	77·0
Average	14·72	3·07	75·7
	Test of differences between means		
D.F.	6	6	6
F.	2·29	2·16	2·89*

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

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Table 1 shows that there is little difference between the means of the populations in the three characters. For body-size and development time the differences between means fell just below and, for survival, just above the 0.05 level of significance.

Turning now to the F_1's and the F_2's we wish to know: (1) the relation of the F_1 to the average of the parents, (2) the differences between F_1 and F_2, since, if there is any heterosis in the F_1 and loss of co-adaptation in the F_2, we might expect significant differences in this comparison, and (3) the comparison of the within-culture variance between parent populations, F_1 and F_2. Tables 2 and 3 set out

Table 2. *Comparisons between the mid-parent and* F_1 *and between* F_1 *and* F_2 *values* ($\times 10^2$) *in crosses between Scottish populations*

	F_1–Mid-parent		F_1–F_2	
Cross	Body-size	Development time	Body-size	Development time
2 × 4	1·9	−1·4	−0·5	1·4
2 × 6	−3·1*	0·1	1·2	1·0
2 × 8	1·1	−2·8*	3·8*	−1·6
4 × 6	−1·0	1·7	1·0	−0·2
4 × 8	−1·3	−2·7*	2·0	0·2
6 × 8	−3·9*	0·8	0·4	−0·7
Average	−1·1	−0·7	1·3	0·0

Approximately 60 flies were scored for each population, F_1 and F_2.
* Indicates significance at the 0·05 level of probability.

Table 3. *Comparisons of pooled with-culture variance* ($\times 10^4$) *between parents,* F_1 *and* F_2 *in crosses between Scottish populations*

	d.f.	Body-size mean square	Development time mean square
Parents	454	140	24
F_1	693	112	23
F_2	644	135	29

the relevant data. When interpreting Table 2 and later tables it should be remembered that if heterosis in the F_1 and decline in the F_2 occur, the estimates: (F_1–mid-parent) and (F_1–F_2) will have opposite sign for body-size and development time respectively, since heterosis is expressed as larger size and shorter development time.

For body-size, two of the F_1's are significantly smaller than the mid-parent size. The others do not depart from the intermediate value. For development time two of the F_1's have a shorter time than the average of their parents, but none of the differences between F_1 and F_2 is significant and the average for the entire series is close to zero. With respect to variances (Table 3) there is no evidence of heterogeneity between corresponding genotypes and so the pooled values provide a valid

comparison. There is no evidence of heterogeneity between the variances of parents, F_1 and F_2.

So we must conclude that there is little evidence of genetic differences between these populations—derived from sites up to 60 miles apart—sufficiently great to produce either a decline in performance or an increased variance in the F_2 from crosses between them.

(ii) *The phenotypic variation of wild flies*

Interpretation of the evidence for or against differences in co-adaptation between populations must take account of the range of environmental variation commonly encountered and how far this is reflected in phenotypic variation, since this will be

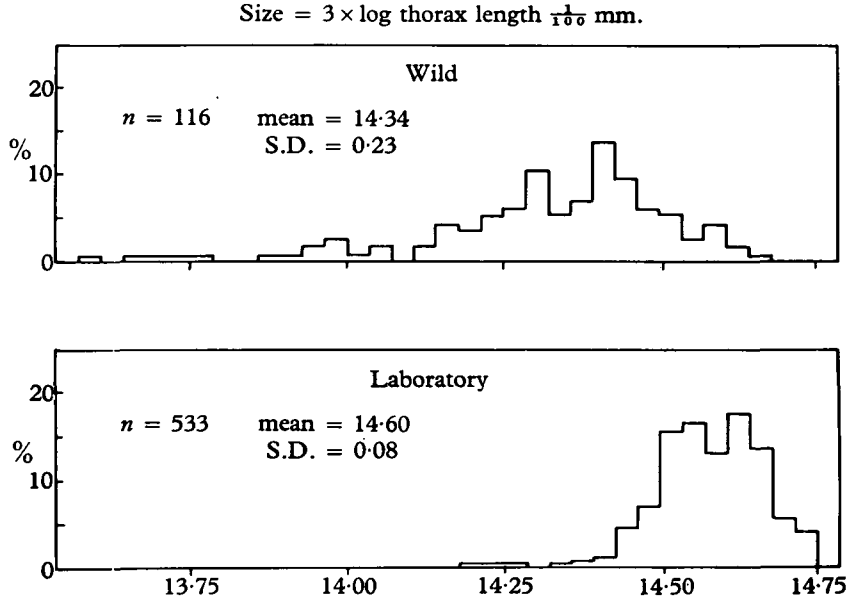

Fig. 2. The mean and variance of wild and laboratory reared flies.

related to the degree to which development is adjusted to ensure stability of phenotype in different characters. Populations and species may well differ in both respects, and ultimately we need to analyse the genetic basis of differences in the degree of stability. As a first step in this direction, we need records of phenotypic variation among wild individuals in different populations and species. Some preliminary data are available for the variation of body-size of wild flies of *D. subobscura* trapped at the Helensburgh site. Samples were caught on several different occasions but, since average size and variability did not differ significantly, they have been combined. The mean and variance of this combined sample is compared in Fig. 2 with the mean and variance of Helensburgh flies grown in the laboratory at 18° C. on the unrestricted live yeast diet.

The average size of the wild flies is appreciably less and the variance considerably greater than that of the laboratory reared flies. The greater variance is associated

with a distribution (which is highly skewed in the direction) of smaller size. Fluctuation in temperature and larval food supply are responsible for this greater variability. Little is known about the temperature of the natural habitat but from the evidence given later on the effects of growing *subobscura* at different temperatures, it is reasonable to attribute most of the observed variation to differences in nutrition. The range of size can be very great; the smallest flies are about one-third of the size of the largest which are about the same size as those grown in the laboratory.

It has been reported by Sokoloff (1957) for *D. pseudoobscura* that the body size of wild flies is comparatively constant and this has been adduced as evidence that food shortage is unimportant in larval life. This is clearly not so for *subobscura*. Also, as stated previously (Robertson, 1961*a*), by virtue of the tendency for body-size to be less affected than development time by inadequate larval diets, such gross variation in body-size almost certainly betokens even greater variation in the duration of larval life. Hence, we may infer that in this population of *subobscura* wide variation in the larval food supply is a regular feature of the environment. It is worth noting here, in view of the common tendency to assume otherwise, that this does not necessarily involve differences in the level of competition experienced by the larvae. It could equally well arise from a diffuse distribution of larvae in an environment in which the quantity or quality of the food supply is often inadequate. Until the natural habitat of the larvae is known, this problem will remain unsolved with the attendant uncertainty as to which experimental conditions are most relevant to those in nature.

(iii) *Comparisons between more widely separated populations*

Since the crosses between the Scottish populations failed to show any evidence of co-adaptation, a greater degree of separation was evidently needed to demonstrate the phenomenon. Therefore populations were obtained from London, Charlottenlund (Denmark), Chur and Zurich (Switzerland) and Qiryat (Israel). The Helensburgh and Israel sites are about 2,500 miles apart.

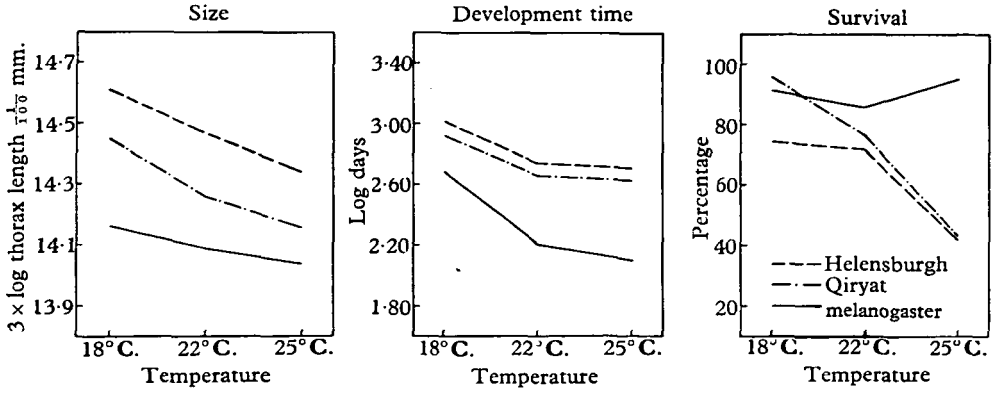

Fig. 3. Body-size, development time and survival of a northern and southern race of *D. subobscura* and a cage population of *D. melanogaster* at three temperatures on the live-yeast medium.

As a preliminary trial, comparisons were carried out on one of the Scottish populations (Helensburgh, site 10) and Qiryat (Israel). The parent populations were grown on the live-yeast medium, on the standard synthetic diet and on a series of protein deficient media to test for gene-environment interaction. Since it is likely that these two populations are adapted to a different range of temperatures, cultures on the synthetic media were set up at both 18° C. and 22° C., while the live-yeast controls were grown at 25°C. as well. In addition, flies from a population of *D. melanogaster* were also grown under these different conditions to provide a general check on experimental procedure, since the nutritional attributes of this *melanogaster* population were already well known. At the same time this enabled us to detect differences between the species in reaction to diet but it is more convenient to consider this particular aspect later.

Table 4. *Average body-size, development time and survival of the Helensburgh and Qiryat populations on unrestricted diet at different temperatures*

	Temperature		
POPULATION:	18° C.	22° C.	25° C.
(a) *Body-size:* 3 × log thorax—$\frac{1}{100}$ mm.			
Helensburgh	14·61	14·47	14·34
Qiryat	14·45	14·26	14·16
Difference	0·16**	0·21**	0·18**
(b) *Development time:* log days			
Helensburgh	3·02	2·74	2·71
Qiryat	2·93	2·66	2·63
Difference	0·09**	0·08**	0·08**
(c) *Survival* (%)			
Helensburgh	74·8	72·0	42·4
Qiryat	96·0	76·8	43·6
Difference	−21·2*	−4·8	−1·2

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

Table 4 shows the comparisons between the two populations on the live-yeast medium. The Israel population is about 16–20% smaller than the northern population and development time is some 10% shorter. This difference is probably correlated with the higher temperature of the environment in view of Prevosti's (1955) evidence of a positive association between wing length and temperature. But oddly enough this is not reflected in the survival figures. Survival at the higher, definitely sub-optimal, temperature of 25° C. is low for both populations and they hardly differ, while at 18° C. the Qiryat population has actually a higher average survival than Helensburgh. A test for gene-environment interaction for the three temperatures on this medium failed to provide evidence of statistically significant

differences in either body size or development time, but showed a small but significant interaction for survival (Table 5).

Table 5. *Test of gene-environment interaction for different characters on two extreme populations reared at three different temperatures on the live-yeast medium*

	Degrees of freedom		Mean square		
Character	Interaction	Error	Interaction	Error	Variance ratio
Body-size	2	234	3·0	2·3	1·3
Development time	2	495	1·5	0·8	1·9
Survival	2	24	56·8	15·0	3·8*

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

For the comparisons on the sub-optimal diets the results are expressed as deviations from performance on the live yeast diet at the corresponding temperatures—18° C. or 22° C.—and these are set out in Table 6. The figures again show a remarkable consistency on the part of the two populations with respect to size and duration of development.

Table 6. *Growth and survival of the two extreme populations, on low protein diets at two temperatures*

	Deviation from performance on live-yeast medium			
	18° C.		22° C.	
% protein in diet	H	Q	H	Q
		Body-size ($\times 10^2$)		
5	−2	−7	−8	−9
3	−9	−13	−17	−20
2	−27	−26	−30	−32
		Development time ($\times 10^2$)		
5	5	7	6	8
3	20	23	25	26
2	36	34	50	50
		Survival (%)		
5	−15	−24	−5	−1
3	−12	−24	−15	−16
2	−51	−77	−42	−58

H and Q refer to the Helensburgh and Qiryat populations.

4. FURTHER COMPARISONS BETWEEN POPULATIONS

To provide a further check on the consistency of performance of different populations, the two extreme and also three additional populations derived from sites near London, Charlottenlund and Zurich were tested on four different diets. These comprised the standard synthetic medium with (*a*) 5% protein, (*b*) 3% protein,

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(*c*) the fructose omitted, and (*d*) the RNA content reduced to one-quarter the usual concentration. Four replicate cultures were set up per genotype per treatment. The test was carried out at 22° C.

The results are shown in Table 7 along with the test of gene-environment interaction. Omission of fructose has no effect on body-size and only a slight lengthening of development time. Reduction of the protein level to 3% decreases body-size by about 5% and lengthens development time by about 13% compared with performance on the control medium. Reduction of the level of RNA to one-quarter the usual concentration reduces body-size further and extends development time by about 20%, but gene environment interaction is virtually absent, even for the duration of development which is notoriously susceptible to nutritional variation.

Table 7. *The reaction of different geographical races to different sub-optimal diets*

Deviations from standard medium

Body-size ($\times 10^2$)

Diet	H	L	C	Z	Q
No fructose	2	0	−1	−1	1
Low protein	−1	−3	−6	−8	−4
Low RNA	−5	−8	−10	−10	−8

Development time ($\times 10^2$)

	H	L	C	Z	Q
No fructose	3	1	5	2	4
Low protein	17	11	13	11	14
Low RNA	22	20	21	19	21

Test of gene-environment interaction

	Degrees of freedom		Mean square	
Character	Interaction	Error	Interaction	Error
Body-size	12	320	1·5	4·7
Development time	12	749	1·4	1·9

H = Helensburgh; L = London; C = Charlottenlund; Z = Zurich, Q = Qiryat.

In view of this regularity in growth, both size and development time have been averaged for all four treatments for the five different populations and this mean provides a reliable estimate of their relative differences; these are listed in Table 8. It should be remembered that on the scale used, a difference of 0.10 in body-size is roughly equivalent to a 10% difference in weight.

There is striking heterogeneity between populations in body-size. The two extreme populations Helensburgh and Qiryat differ by some three standard deviations of the variance between individuals within cultures. The other populations are at intermediate levels and there is a general decrease in size from north to

Table 8. *Body-size and development time of different geographical races, averaged over four treatments—log scale*

Population	Body-size	Development time
Helensburgh	14·34	2·88
London	14·24	2·84
Charlottenlund	14·29	2·84
Zurich	14·16	2·85
Qiryat	14·15	2·82
Average standard error	0·02	0·01

south, correlated no doubt with the prevailing temperature. It will be recalled that Prevosti (1955) claimed there was a correlation between wing length and the July isotherm. This particular relationship was not tested in the present data, since accurate information about temperature variation was not available, and also because the July isotherm is perhaps a rather arbitrary measure of the temperature of the habitat. Scottish *subobscura* occur most abundantly in September and October, while in Israel they are abundant only in late April and early May.

Development time showed much less evidence of association with the general temperature of the habitat, although the largest and smallest populations had also the longest and shortest development time.

The next step in the analysis was to test for evidence of co-adaptation in crosses between populations from geographically isolated sites. Before considering this analysis however, we must determine whether the performance of the F_1 is influenced by the direction in which the cross is carried out, that is, whether there is any evidence of maternal effect. Progeny of the reciprocal crosses between the two extreme populations Helensburgh and Qiryat were included in the tests on the four

Table 9. *A test of difference between reciprocal crosses and deviation from the mid-parent value* ($\times 10^2$)

	Difference between means of reciprocals—(H × Q) − (Q × H)					
Diet	Body-size	*t*	Degrees of freedom	Development time	*t*	Degrees of freedom
Standard medium	−2·9	1·4	54	1·2	1·3	113
No fructose	2·8	0·9	46	0·7	0·6	81
Low protein	−2·7	1·1	46	−2·1	1·7	97
Low RNA	−1·8	0·4	34	−0·9	0·4	51
	Average of crosses—Mid-parent					
Standard medium	−1·6	1·2	100	0·4	0·6	191
No fructose	2·8	1·6	108	−1·7	2·3*	197
Low protein	1·0	0·5	92	−0·4	0·5	202
Low RNA	−1·8	0·8	94	−2·5	1·8	141

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

different kinds of medium, which have just been described. The relevant data are summarized in Table 9 and they show the result of testing the significance of difference (*a*) between the reciprocals and (*b*) between the average of the reciprocals and the mid-parent value for body-size and development time. There is only one statistically significant difference between reciprocals and when the average of the crosses is compared with the mid-parent values the F_1 turns out to be almost exactly intermediate between the parents. Hence maternal effects can be ignored.

5. COMPARISONS OF MID-PARENT F_1 AND F_2

Although the F_1 in the cross between the most widely separated populations is intermediate there is still the possibility that the F_2 from such crosses may reveal loss of co-adaptation. To test this and provide further comparisons, flies from the two most extreme populations Helensburgh and Qiryat and also from the intermediate Swiss population (Chur) were intercrossed and taken to an F_2. As usual the experiment was arranged so that parents F_1 and F_2 were all set up together on the same batch of medium. Since the previous tests failed to show gene-environment interaction with respect to diet, there seemed little point in growing flies on more than one type of medium in this test and so flies were grown only on the ordinary live-yeast medium at 22° C. By reducing the number of treatments the size of the experiment could be increased. For each parental, F_1 and F_2 series, seven replicate cultures were set up and ten females were scored from each culture wherever possible. Each cross was made reciprocally.

Table 10 shows the mean sizes for the two characters in the three populations. As usual the extreme populations show a striking difference in body-size and development time—Helensburgh flies are some 15% larger than Qiryat ones—but the Swiss population closely resembles the Israel one. The Swiss population is just significantly larger than the latter at the 0.05 level, but for development time there is no significant difference.

Table 10. *Comparison between three parental populations grown on live-yeast medium—log scale*

Population	Body-size	Development time
Helensburgh	$14{\cdot}32 \pm 0{\cdot}009$	$2{\cdot}754 \pm 0{\cdot}005$
Chur	$14{\cdot}19 \pm 0{\cdot}009$	$2{\cdot}713 \pm 0{\cdot}004$
Qiryat	$14{\cdot}17 \pm 0{\cdot}008$	$2{\cdot}714 \pm 0{\cdot}004$

With respect to reciprocal differences the Qiryat and Helensburgh cross shows no difference as before, and the same is true for the cross between Qiryat and Chur, but for Chur and Helensburgh there was a clearly significant difference for both body-size and development time (Table 11). This difference was not correlated with the phenotype of the female parent since the F_1 of the cross (Helensburgh female × Chur

Table 11. *Reciprocal differences in crosses between Helensburgh, Chur and Qiryat populations* ($\times 10^2$)

	Body-size		Development time	
Comparisons	$M_1 - M_2$	t	$M_1 - M_2$	t
$(Q \times C) - (C \times Q)$	0·6	0·44	−1·2	1·98
$(Q \times H) - (H \times Q)$	0·6	0·50	0·6	1·20
$(H \times C) - (C \times H)$	−2·7	2·53**	−3·4	4·74**

H, C and Q refer to the Helensburgh, Chur and Qiryat populations. The male parent is referred to first in the list of crosses.

** indicates significance at the 0·01 level.

male) was smaller and developed faster than the reciprocal. The faster development time makes it unlikely that this difference reflects cultural variation which might have been more unfavourable for this group of cultures, so perhaps an interaction between zygote and cytoplasm is involved.

The F_1's from crosses between Chur and Qiryat and Qiryat and Helensburgh have been averaged, but, in the other cross, the two reciprocals are listed separately in Table 12, which sets out the relevant comparisons. Apart from the maternal effect

Table 12. *Comparisons between mid-parent values* F_1 *and* F_2 ($\times 10^2$) *of the crosses between the widely separated populations*

Cross	F_1–M.P.	F_2–M.P.	F_1–F_2
	Body-size		
Q × C	−0·9	−0·5	−0·4
Q × H	−0·9	−1·1	−0·2
C × H	1·0	0·5	0·5
H × C	−1·8	0·5	−2·2*
	Development time		
Q × C	−0·4	−0·1	−0·2
Q × H	−0·8	−1·1	−0·3
C × H	−0·5	0·2	−0·6
H × C	−3·9**	0·2	−4·0**

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

just noted, the F_1 values do not depart from the mid-parent values, nor do any of the F_2 means differ significantly from the mid-parent for either character. So here again there is no evidence whatever of a decline in performance of the F_2. The only significant difference between F_1 and F_2 is accounted for by the apparent maternal

effect. Finally, there are the comparisons of variance which are shown in Table 13. They reveal a striking homogeneity in the comparisons between the parent populations and between parents and either F_1 or F_2.

Table 13. *The within-culture variance of parental populations, and* F_1 *and* F_2 *of crosses* ($\times 10^4$)

	Q × C		Q × H		H × C	
	d.f.	Mean square	d.f.	Mean square	d.f.	Mean square
			Body-size			
Parents	126	128	126	162	126	118
F_1	126	138	126	149	108	166
F_2	126	153	126	172	108	141
			Development time			
Parents	223	32	235	28	204	37
F_1	259	34	227	30	201	32
F_2	103	37	106	30	56	44

Dr Maynard Smith has informed us that in studies on body-size in a Scottish and Israel strain of *subobscura* he found that parents F_1, F_2 and backcrosses showed additive behaviour as in our tests, but in his case there was some evidence for a greater variance in the F_2. If many genes distributed over all chromosomes are responsible for the phenotypic differences between the parents, increased variance of the F_2 relative to parents or F_1 would be difficult to detect. But if, in the F_2 of wide crosses there is a general lowering of the level of co-adaptation, then we might expect an increase in variance due to the enhanced importance of novel and often unfavourable gene combinations. But there is no evidence of this, even in development time, which, as a sensitive indicator of heterosis, might be expected to display such effects more readily than body-size. So the comparisons of variability support the conclusions drawn from the comparisons between the means—there is no evidence for differences in co-adaptation even in crosses between populations from the northern and southern extremities of the range of the species.

6. AN ALTERNATIVE TEST OF CO-ADAPTATION

Since the crosses fail to provide evidence of loss of co-adaptation or balance, an alternative approach was used following the suggestions of Breese & Mather (1960). These authors stated that 'interpopulation hybrids should show a greater spread round their overall mean, which itself need not depart from the mean of intrapopulation families. This spread must be measured as the variance of the mean of families reared from interpopulation crosses.' This interesting suggestion arose in a discussion of the behaviour of chaeta number and so it was decided to test it on this character.

The populations Helensburgh and Qiryat were chosen for these comparisons since they were most widely separated geographically, have been shown to differ greatly in average body-size and development time and will have an entirely different pattern of inversions (Goldschmidt, 1958). So there is independent evidence of extensive genetic variation between them and they are therefore ideally suited for the present purpose. Thirty pairs were mated at random from each of the two populations to provide altogether sixty intra-population families. Thirty matings were also made between flies chosen from the two different populations. In half of these the males were drawn from the Helensburgh population and the females from Qiryat, while in the other fifteen the cross was made the other way round.

The female of each pair was allowed to lay eggs for two days in a vial of the maize meal medium and then removed to minimize the risk of overcrowding. All the F_1 were allowed to emerge and all the females were scored, generally twenty or more per family. The sternital bristles of the 3rd and 4th abodminal segment were counted. It is well known that sternital bristle number is much less affected by variation in diet than body-size. Other tests on the same populations, under similar favourable conditions, failed to demonstrate any evidence of heterogeneity between cultures for this character, so the use of one culture per family is unlikely to confuse genetic with environmental differences to any important degree. Since there was no evidence of reciprocal differences the direction of the cross was ignored. The average number of bristles per segment in the two populations is closely similar, 18·75 for Helensburgh, 18·08 for Qiryat—a small, but statistically significant difference. The average of the F_1's worked out at 18·55 close to the mid-parent value, so, in spite of their differences in general size and development time, these two populations have about the same bristle number.

Table 14. *Within and between family variance of bristle number in intra- and inter-population mating*

	Mean Square		Degrees of freedom			
Matings	Within families	Between families	Within families	Between families	Components	σ_F^2
Helensburgh	8·87	66·07	505	29	$\sigma^2 + 18\sigma_F^2$	3·18
Qiryat	7·47	100·88	839	29	$\sigma^2 + 29\sigma_F^2$	3·22
Cross	7·99	70·57	754	28	$\sigma^2 + 25\sigma_F^2$	2·82

σ_F^2 refers to the between family component of variance.

Table 14 shows the variance analyses for the sum of the bristle counts on the two segments. The within-family variance is the same for all three groups. There is highly significant heterogeneity between families, but the between-family components of variance, weighted to allow for variation in the number of individuals in families, work out at 3·18 and 3·22 for the Helensburgh and Qiryat matings and 2·82 for the cross. So there is not even a suggestion of greater spread about the overall mean in the cross between these widely different populations. Thus this different

kind of test agrees with the earlier ones in failing to demonstrate evidence for co-adaptation.

These figures also provide estimates of heritability. Assuming that the variance between families represents half the additive variance and that the variance within families is made up of half the additive plus the environmental variance, we can estimate the ratio of genetic to total variance as respectively 0·53 for Helensburgh and 0·60 for Qiryat which are the kind of values we should expect for a character like bristle number.

7. THE EFFECTS OF LONG-TERM LABORATORY CULTURE

Seasonal fluctuation in the abundance of wild flies and the need to expand populations for a generation or so to obtain adequate numbers made it impossible to use freshly caught wild flies or their immediate descendants for all the tests which we have described, and it could be argued that culture in the laboratory has led to differences in selection pressure so that the flies used in these experiments are not typical of the wild populations from which they were derived. Although large numbers were kept to minimize inbreeding, significant changes in the genetic structure of populations might well have occurred in the course of twenty generations.

Since differences in larval diet probably represent the major differences between environments the performance of the Helensburgh population, which has been used repeatedly during these tests, was compared with that of another sample taken in October at exactly the same site two years later. After one generation of expansion the two populations were compared on four different diets, comprising either the standard synthetic medium or modifications in which fructose was omitted, or protein or RNA was reduced to the same level as in the comparisons between the different geographical races. The experiment was run at 18° C.

Table 15. *Comparisons of performance between wild and laboratory populations from the same locality*

	Differences between means (wild − laboratory; $\times 10^2$)			
	Body-size		Development time	
Diets	Difference	Significance level	Difference	Significance level
Standard	0·9	$p > 0·1$	−2·8	$p > 0·1$
No fructose	−1·1	$p > 0·1$	−2·5	$p > 0·1$
Low protein	−1·2	$p > 0·1$	−2·7	$p > 0·05$
Low RNA	−1·4	$p > 0·1$	0·8	$p > 0·1$

As noted earlier in Table 7 such treatments, especially ones with low protein or low RNA, reduce body-size and lengthen development time. But Table 15 shows that there is no significant difference between the wild and the laboratory populations on any of the diets, nor is there any evidence of consistent differences in the within culture-variance which might suggest differences in adaptation. So although this test does not exclude other undetected differences we may reasonably assume

that the lack of evidence for co-adaptation is a true reflection of the genetic properties of the populations concerned and is unconnected with possible changes due to laboratory culture.

8. THE EFFECTS OF INBREEDING IN TWO SPECIES OF DROSOPHILA

(i) *General*

The conclusion that loss of co-adaptation is unimportant in crosses between populations of *D. subobscura*, whereas it has been claimed to occur in other species, raises the problem whether such apparent contrasts are related to differences in breeding structure of the species concerned. A widely distributed species like *subobscura* may be an extreme out-breeder and the fluctuation in gene arrays may be ordinarily so great that wide crosses do not impair the stability of growth rates and general metabolism.

One way of comparing species with different breeding structures is to measure their response to inbreeding. Out-breeders should suffer a greater decline than those in which consanguineous matings are sufficiently more frequent to influence the genetic structure of the species generally. Ideally we should prefer to compare species with well-defined differences in population structure. Since such information was lacking for another suitable species we decided to compare the response to inbreeding in populations of *D. subobscura* and *D. melanogaster*. In the latter species the foundation population was a long-established cage population descended from a number of wild individuals, whereas in *D. subobscura* the inbred lines were derived directly from wild populations. Apart from possible fixed differences between the species arising from intrinsic differences in population structure in nature, the *D. melanogaster* population could be regarded as approximating more closely to one with restricted opportunities for out-breeding compared with typical *D. subobscura* populations and so the response to inbreeding of the *melanogaster Pacific* population and the two *subobscura* populations Helensburgh and Qiryat, should indicate whether these populations are qualitatively different in their reaction to inbreeding Since the effects of inbreeding were compared by growing flies on several different diets, we have first to see whether the two species differ in their growth under similar nutritional conditions.

(ii) *Growth comparisons between* D. melanogaster *and* D. subobscura

As noted earlier, in the comparisons between Qiryat and Helensburgh on the live-yeast medium at 18, 22 and 25° C. and on diets with progressively lower levels of protein at temperatures 18° C. and 22° C., cultures of *D. melanogaster* were set up at the same time. Behaviour of the three series is shown in Fig. 3. The main points to note are as follows. (1) On the live-yeast medium *melanogaster* is able to survive at a higher temperature than either the northern or southern races of *subobscura*. At 18 and 22° C. there is little difference between the species in survival, while at 25° C. *subobscura* is greatly reduced, whereas *melanogaster* is unaffected. (2) *D. melanogaster* has a smaller size and a shorter development time than even the

Qiryat population and the difference in development time is proportionately greater than the difference in body-size. Although both species decline in size as the temperature increases, the proportional decline is greater for *subobscura* than *melanogaster*. But for development time the position is reversed. Thus, at 18° C. the difference between the species is least for development time and greatest for body-size. These relations are shown in Fig. 3. Such differences no doubt reflect differences in the average temperatures to which the species are adapted although the relationships are evidently complex. If we assume that 25° C. and 18° C. are close to the optimal temperature for *melanogaster* and *subobscura* respectively, then the former responds to lower temperatures by a relatively large change in development time and comparatively small change in body-size, while in *subobscura* the situation is reversed for the two characters.

In the comparisons of growth at different levels of protein at 18° C. and 22° C. there is a remarkable similarity in general performance between the two species for either body-size or development time, and this reflects the differences on the live-yeast medium just discussed. But there are two important differences. Firstly, *D. subobscura* is unable to survive as well as *melanogaster* at the lower levels of protein, and the difference is especially striking at 22° C. Secondly, when the slower growth on 5% protein is compared with performance on live yeast, *melanogaster* is relatively more delayed on the synthetic diet than either of the *subobscura* populations. The difference is more marked at 22° C. than 18° C. The synthetic medium is not as favourable as live yeast for rapid growth, but as the temperature declines the effective difference between the two media diminishes. Such differences between the species in reaction to environment must be taken account of if we are to choose the most effective conditions in which to compare the effects of inbreeding on the two species.

(iii) *The effects of inbreeding*

A series of inbred lines were set up from two *subobscura* populations Qiryat and Chur, and the *Pacific melanogaster* population. Comparisons were carried out after seven generations of brother-sister mating, equivalent theoretically to some 90% loss of heterozygosity. Seven lines were available for the Chur and *melanogaster* and six for the Qiryat series. Samples from all the lines and the three foundation populations were set up at the same time on alternative diets which comprised the live-yeast medium and also synthetic media in which either the protein was reduced to 3% or the RNA was reduced to one-quarter the usual concentration, or the level of choline was deficient. In the latter case lecithin was omitted from the synthetic medium and 80 μg. of choline added per culture. Sang (1956) showed that about 200 μg. of choline per culture is adequate for *D. melanogaster* and that reduction below this level increases development time and diminishes body-size. The test was carried out at 18° C. For each genotype and treatment there were five replicates on the live medium, four on the sterile medium. Where available, eight females per culture were measured, while development time was scored for all females which hatched. Tables 17, 18 and 19 summarize the results for body-size, development

time and survival. The results can be considered under two headings: (*a*) The deviations of the inbred lines from the foundation populations on the different media and (*b*) the within-culture variance of the inbred lines relative to that of their foundation population.

Table 16. *The effects of rearing the non-inbred populations on alternative diets*

Deviations from performance on the live-yeast medium

Genotypes	Low protein	Low RNA	Low choline
	Body-size ($\times 10^2$)		
D. melanogaster	−14**	−21**	−53**
D. subobscura			
Chur	−21**	−15**	−14**
Qiryat	−31**	−18**	−14**
	Development time ($\times 10^2$)		
D. melanogaster	19**	30**	36**
D. subobscura			
Chur	24**	29**	11**
Qiryat	24**	21**	8**
	Survival (%)		
D. melanogaster	−1	−3	1
Chur	−46**	−25	−1
Qiryat	−56**	−22**	−10

** indicates 0·01 level of significance.

Before considering the data in this order, certain further differences between the species must be noted. These are shown in Table 16, which expressed the mean values of the different characters of the non-inbred foundation populations as deviations from the corresponding means on the live-yeast medium. As noted above, *subobscura* is less able to withstand a reduction in protein content than *melanogaster*. This is indicated by the relatively greater reduction in body-size, greater delay in development time and especially by the drastic reduction in survival which is reduced by about 50% in *subobscura* but is unaffected in *melanogaster*. With respect to lower RNA levels, the two species are more or less alike for body-size and development time, but *subobscura* shows lower survival than *melanogaster*. There is a further well-marked difference between the species in their reaction to lower levels of choline. In *melanogaster* body-size is drastically reduced and development time extended, although survival is unaffected. But in *subobscura* body-size and development time are only moderately affected and only in Qiryat is there some evidence of a reduction in survival. So, the requirements for choline are considerably higher in *melanogaster* than *subobscura*.

Turning now to the comparisons between the arrays of inbred lines, we find, as expected, that body-size is reduced, development time lengthened and survival lowered. Table 17 shows that the average reduction in body size in these three

groups of inbred lines is about the same on the unrestricted diet, low protein and low RNA. In the Qiryat group on low protein, the inbreds are, on average, larger than the controls but this is almost certainly an artefact due to the latter being atypically smaller. As we have seen above, a low protein diet is especially unfavourable for *subobscura* and small differences between cultures, possibly due to uncontrollable differences in heat exposure during autoclaving, may account for this. Reduction in the level of RNA enhances the effects of inbreeding for both

Table 17. *The effects of inbreeding on body-size in the Pacific populations of* D. melanogaster *and Chur and Qiryat populations of* D. subobscura

Deviations from foundation population ($\times 10^2$)

		Alternative diets			
Genotypes		Yeast medium	Low protein	Low RNA	Low choline
D. melanogaster	Line No. 1	−7**	−8**	−9**	−16**
	2	−13**	−15**	−25**	−7
	3	−4	−8*	−4	−2
	4	−5*	−2	−5	−17**
	5	−6**	−7**	−13**	−8
	6	−11**	−12**	−16**	−43**
	7	−6**	−2	−11**	−10*
D. subobscura					
(*a*) Chur	Line No. 1	−4*	—	—	−10*
	2	−13**	—	−20**	−21**
	3	1	0	−6	+7*
	4	−5**	−14**	−10	−7
	5	−5**	−16**	−2	−13**
	6	−10**	0	−12*	0
	7	−6**	−7	−7	−4
(*b*) Qiryat	Line No. 1	−2	9	−19**	−8**
	2	1	—	−17**	1
	3	−2	—	−3	−6
	4	−10**	6	−21**	−32**
	5	−2	7	−3	−3
	6	4*	−1	3	1
		Average deviations			
D. melanogaster		−8	−8	−12	−14
D. subobscura					
Chur		−6	−8	−15	−7
Qiryat		−2	5	−10	−9

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

species and this is in line with the similar evidence derived from other inbred lines of the *Pacific* population reported by Prabhu & Robertson (1962). On low choline the lines of *melanogaster* are relatively more reduced than those of *subobscura* and

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this is almost certainly related to the species difference in the reaction to sub-optimal levels of choline we have already noted.

Table 18. *The effects of inbreeding on development time in the Pacific population of* D. melanogaster *and the Chur and Qiryat populations of* D. subobscura

Genotypes		Deviations from the foundation population ($\times 10^2$) Alternative diets: Yeast medium	Low protein	Low RNA	Low choline
D. melanogaster	Line No. 1	7**	−2*	−7**	−2
	2	5**	4**	12**	15**
	3	10**	−2*	0	7**
	4	2**	1	7**	6**
	5	2**	4**	2	4**
	6	14**	6**	−2	12**
	7	−1*	−1	−2	0
D. subobscura					
(*a*) Chur	Line No. 1	3**	—	—	2
	2	11**	—	4	10**
	3	4**	12**	3	5**
	4	2*	13**	13**	2
	5	11**	21**	3	11**
	6	1	10**	2	0
	7	8**	12**	0	3**
(*b*) Qiryat	Line No. 1	7**	−4	16**	9**
	2	10**	—	17*	5**
	3	2	—	2	7**
	4	15**	4	13**	11**
	5	13**	13**	14**	8**
	6	8**	19**	4*	3*
		Average deviations			
D. melanogaster		6	1	1	6
D. subobscura					
Chur		5	14	10	4
Qiryat		10	8	10	7

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

For development time (Table 18) the average extension is about the same for the three groups on unrestricted diet and low choline, but on low protein and low RNA the *subobscura* lines are more adversely affected than the *melanogaster* lines. With respect to survival (Table 19) there is little average difference on either the unrestricted diet, or the medium which is deficient in RNA. On low protein the *melanogaster* and Chur averages are the same, while the Qiryat series has a higher survival than the controls, probably for the same reason as those suggested for the comparable discrepancy for body-size. On low choline the effect of inbreeding is

relatively greater for *melanogaster* than *subobscura*, and no doubt this again reflects the species difference in reaction to low levels of this nutrient. Thus, there is little evidence of a consistent difference between the species in the average effects of inbreeding on these three characters if we allow for the species differences in reaction to either low protein or low choline.

Table 19. *The effect of inbreeding on survival in the population of* D. melanogaster *and two populations of* D. subobscura

		Deviations from the foundation population (%)			
		Alternative diets			
Genotype		Live yeast	Low protein	Low RNA	Low chloine
D. melanogaster	Line No. 1	−8	−1	−4	−10
	2	−25**	−22*	−41*	−64**
	3	−6	2	−10	−19
	4	−29	−26*	−32**	−45**
	5	−8	−2	−11	−19
	6	−42**	−44**	−46**	−64**
	7	−44**	−23*	−27**	−36*
D. subobscura					
(*a*) Chur	Line No. 1	−39**	—	—	−24*
	2	−31*	—	−32	−36**
	3	−27*	−15	−36*	−19
	4	−20*	−19	−36*	−40**
	5	−30*	−19	−22	−30*
	6	−43**	−14	−25	−26
	7	−7	−24	−2	−5
(*b*) Qiryat	Line No. 1	−20**	17	−30	−4
	2	−24*	—	−30	−14
	3	−24**	—	−17	−18*
	4	−40**	16	−27	−12
	5	−26**	−10	−26	−22**
	6	−24**	7	−7	−17
		Average deviations			
D. melanogaster		−24	−17	−25	−37
D. subobscura					
Chur		−29	−18	−25	−27
Qiryat		−27	7	−23	−15

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

Finally, there are the comparisons of the within-culture variance which are set out in Table 20 as a ratio of the standard deviation derived from the pooled variance of the inbreds to that of the standard deviation of the non-inbred populations on the alternative diets. It is a striking fact that in almost all cases this ratio exceeds unity for the two characters. From past experience of growth on the live-yeast medium and from the homogeneous nature of the agar gel in the synthetic diets,

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there is little doubt that the within-culture variance is predominantly genetic. Hence, the substantial loss of heterozygosity which must have accompanied seven generations of inbreeding is not reflected in lower variability but often in an increase over that of the highly heterozygous populations. This probably reflects, to a large extent, the enhanced effects of segregation on the phenotype due to relatively greater importance of gene-gene and gene-environment interaction which has been shown regularly to accompany increased homozygosity (Robertson, 1959, 1962).

Table 20. *The ratio of the pooled within-culture variance of the inbred lines to that of the foundation populations*

	Alternative diets			
	Live yeast	Low protein	Low RNA	Low choline
		Body-size		
D. melanogaster	0·69	1·51	0·59	1·62
D. subobscura				
Chur	1·28	1·64	1·71	0·88
Qiryat	0·98	1·78	1·19	2·88
		Development time		
D. melanogaster	2·62	1·61	1·08	1·27
D. subobscura				
Chur	1·92	0·90	1·09	0·76
Qiryat	2·92	3·93	1·22	1·98

9. DISCUSSION

The chief conclusion from these experiments is that evidence for co-adaptation is entirely lacking. For a variety of different sub-optimal diets and different temperatures we have found neither heterosis nor 'luxuriance' in the F_1, nor decline in performance in the F_2 nor any increase in the phenotypic variance of the latter. The data failed to confirm the existence of relational balance with respect to abdominal bristle number, since the variance between families obtained by crossing different populations is no greater than that between families from the same population. Hence, the original aim to relate the spatial separation between populations to the level of differences in co-adaptation between them has not been realised. But, on the other hand, the data provide a contrast with what has been claimed for other species of *Drosophila*, and these apparent differences must now be considered.

Co-adaptation has been defined (Wallace, 1953) as the mutual adjustment of interacting alleles within the gene pool of a Mendelian population, brought about (*a*) by selection of heterozygous genotypes possessing superior adaptive values or (*b*) by the occurrence of genetic recombination between successive generations. Many authors (Wallace, 1955; Brncic, 1954; Prevosti, 1957; Vetukhiv, 1954) agree that recombination will disrupt balanced gene complexes and lead to lower performance in the F_2. There is not the same agreement with respect to apparent

heterosis in the F_1. Mather (1943) suggested that such heterosis is tangible evidence of differences in balance between the parents and does not necessarily imply superior fitness. Vetukhiv (1957) took such heterosis at its face value as evidence of superior fitness and since its origin, in his experiment, could hardly lie in co-adaptation, heterozygosity *per se* played a major part. Wallace (1955) chose a middle road and attributed such heterosis or 'luxuriance' (Dobzhansky, 1950) to 'heterozygosity for different but integrated gene systems'. Evidently all tastes are catered for by such alternative interpretations.

Vetukhiv's work with *D. pseudoobscura* (1953, 1954, 1956, 1957) is often quoted as evidence for heterosis in the F_1 and breakdown in the F_2 in crosses between populations and so it requires careful examination. The 'natural' populations were constructed by intercrossing a number of strains each descended from single impregnated females caught in the same district (Vetukhiv 1954). Apparently these strains had been kept in the laboratory for a number of generations before use, so they may have been subject to some degree of inbreeding and perhaps also some adaptation to laboratory conditions. The F_1 'between-sites' was created in a similar way except that the males and females were drawn from strains caught in different districts. Hence, both intra- and inter-district matings were between populations subject to an indeterminate degree of inbreeding and both may have been expected to show heterosis, relative to the individual parent strains whose performance is unknown. But previous inbreeding appears to be irrelevant to the comparison between the F_1's from within-district and between-district matings and the origin of this difference remains obscure. The F_2 decline cannot be interpreted without comparable data from "within-site" matings. It may be noted that in similar comparisons of survival for *D. willistoni* and *D. paulistorum*—the former behaved like *D. pseudoobscura* while the latter showed less evidence of F_1 superiority in between-district crosses, so there was suggestive evidence here of a difference between species.

It is perhaps worth noting that in the tests on longevity (Vetukhiv, 1957) the relative superiority of F_1 to F_2 was influenced by temperature, suggesting that the expression of heterosis may be altered by this variable. It is worth recalling here the classic experiments of Dobzhansky (1949) in which the frequency of different inversions was followed in population cages at two temperatures. At the higher temperature equilibrium was attained due to superiority of the inversion heterozygotes, but at the lower temperature there was no evidence of differences in fitness of the alternative genotypes. Hence, the most convincing evidence for co-adaptation, based on characteristic changes in the frequency of particular inversions, when combined with different genetic backgrounds, appears to have been carried out at temperatures which could be regarded as sub-optimal (Spassky, Spassky, Pavlovsky Krimbas, Krimbas & Dobzhansky, 1959). And this prompts the question as to which temperature or range of fluctuation provides the most valid basis for extrapolating to natural conditions.

Perhaps the apparent discrepancies between *D. subobscura* and *D. pseudoobscura* are due not so much to the nature of the tests but to qualitative differences between the species, due to differences in breeding structure, especially differences in average

effective population size and degree of adaptation of different populations to different environments. For the reasons noted earlier, nothing very useful can be said on this score except that both are widespread and generally abundant where they occur. We have seen, in the records of body-size in wild flies, that *subobscura* may be normally exposed to rather drastic nutritional variation and, naturally, the greater the magnitude and severity of such variation the less likely the degree of genetic integration.

The two species resemble each other in the high incidence of inversion polymorphism. Goldschmidt (1956) has demonstrated that Israel populations are clearly differentiated in the frequency of particular inversions from Central and West European populations and there is no doubt that Israel and Scottish populations differ in this respect (Knight, 1961). It was hoped that comparisons of the response to inbreeding between two populations of *subobscura* and a cage population of *melanogaster* might indicate differences in population structure, since the latter population could be regarded as one free from immigration for many generations and was descended from many fewer individuals than would be true for any natural population of *subobscura*. Furthermore, inversions are rare in populations of *D. melanogaster*, and in the *Pacific* population were known to be absent. But these comparisons failed to indicate any major differences except for certain diets which were deficient in particular nutrients for which the two species differ in minimal requirements. Thus, for *melanogaster* the effects of inbreeding were relatively more marked on low choline diets, while the effects of inbreeding, especially with respect to survival, were greater on low protein diets in *subobscura*. Such differences in nutritional requirements between species suggest that more extensive comparisons with other species would be rewarding and might suggest differences in ecology. It is possible, of course, that less intense inbreeding might have shown up differences or, perhaps *melanogaster* and *subobscura* are basically too alike in breeding structure to demonstrate differences in response to inbreeding.

Our experience with the two populations of *subobscura* suggests a less drastic response to inbreeding than that reported by other workers with this species, especially in comparisons on the unrestricted live-yeast cultures. Thus Maynard Smith, Clarke & Hollingsworth (1955) reported a drastic reduction of productivity in five lines after five generations of brother-sister mating. We have been informed by Dr Maynard Smith that this difference probably reflects the relatively less favourable food supply in his experiments than in ours.

Since it has proved impossible to demonstrate differences in co-adaptation from the performance of the F_1 or F_2 crosses between populations as widely separated as those from Scotland and Israel, which differ by about 20% in body-size, for either this character or survival or the duration of development, which is notoriously susceptible to adverse environmental and genetic conditions, it is not in the least surprising that there was also no evidence for relational balance in chaeta number, whose genetic behaviour is known to display much less evidence for gene interaction, generally, than do components of fitness (Breese & Mather, 1960).

It is well established from earlier work (Robertson & Reeve, 1954; Robertson,

1959, 1960, 1961) that the stability of performance and the restriction of the harmful effects of segregation in genetically variable populations involves extensive epistatic interaction. Inbreeding leads, especially for characters associated with fitness, to increased effects of segregation either by gene-gene or gene-environment interaction. The regular tendency for the within-culture variance in the inbred lines of either species to exceed that of the foundation population, provided further confirmation of this general rule. Crosses between inbred lines generally resemble more or less closely the average of the foundation population from which they were derived and the evidence for gene-environment interaction is sharply reduced (Prabhu & Robertson (1962*b*)). Hence, unless special care is taken in the perpetuation and sampling of stocks it is easy to confuse the effects of inbreeding and its dissipation with phenomena peculiar to the recombining of gene arrays from different populations. Effects of this nature will appear more dramatic under stress conditions, including unfavourable larval nutrition and, perhaps especially, higher temperatures.

It appears that growth is well enough buffered within *subobscura* generally,that crossing different populations, which certainly differ at many loci and which have different combinations of inversions, is insufficient to impair growth to any noticeable degree in the F_2. This species may be what Mayr (1959) has termed a 'wide-open' one in which there is a high incidence of immigration, which, coupled with extensive ecological variation, diminishes the likelihood of a high degree of co-adaptation within populations. If gene arrays within populations are not so nicely adjusted as has been commonly supposed there will be greater scope for the spread of favourable genes and gene combinations via immigration from the population in which they arose.

Clearly, the data relating to the genetic properties of populations of *subobscura* suggest that the whole question of effective differences between populations in this and other species is urgently in need of systematic analysis. Such differences must be demonstrable in crosses between populations rather than in the effects of chromosome substitution and manipulation, which, as Mayr (1959) has suggested, may often represent special situations not encountered in nature. Only then can we assess how general or otherwise are the properties we have described for *subobscura*.

10. SUMMARY

1. The paper described an attempt to see whether differences in co-adaptation between populations of *Drosophila subobscura* are related to the distance between them. The mean and the variance of body-size, development time and survival were recorded on parent populations and the F_1 and F_2 of various crosses to test for heterosis in the F_1 and decline in performance or greater variance in the F_2, which might indicate the break-up of co-adapted gene arrays. Comparisons were carried out at different temperatures and on a variety of larval diets, especially sub-optimal ones in which the larvae were grown on synthetic media. A large number of wild flies were caught at sites separated by about 10 miles along a transect of southern

Scotland; these comprised one series of comparisons. For more distant crosses flies were caught at sites in southern England, Denmark, Switzerland and Israel.

2. There were well-defined differences in body-size, and, to a lesser degree, development time between populations from more widely separated localities and these showed evidence of a cline, northern populations having larger body-size. The difference in size between the Scottish and Isreal populations is about 20%.

3. There was no evidence of differences in co-adaptation between populations even in crosses between populations from sites as far apart as Scotland and Israel. The F_1's were always close to the mid-parent values and there was no evidence of breakdown in the F_2 nor of increased variability.

4. There was hardly any evidence of gene-environment interaction either with respect to different diets or to different temperatures.

5. Records of body-size on flies caught in the wild showed that they are extremely variable, indicating great variation in larval nutrition. Under natural condition stability of growth in body-size is conspicuously lacking in this species.

6. An additional test of co-adaptation was based on the between-family variance of abdominal bristle number of intra- and inter-population matings in the two most widely separated populations. There was no evidence of greater variance in the inter-population series.

7. To test for possible differences in breeding structure, the response to inbreeding was determined for two widely separated populations of *D. subobscura* and a long-established cage population of *D. melanogaster*, on an unrestricted larval diet and also on several different kinds of sub-optimal diets. There was little or no sign of consistent differences between the species in their response to inbreeding.

8. This test revealed differences between the two species in their minimum requirements for particular nutrients. *subobscura* is less able than *melanogaster* to withstand lower levels of protein and survival is particularly reduced. On the other hand, *melanogaster* has a considerably higher requirement for choline. Where there are apparent differences between the species in the average effect of inbreeding, the inbreeding effect is greater on the relatively more sub-optimal diet.

9. Comparison of the performance of the immediate descendants of wild flies with those derived from the same site, but kept in the laboratory for some twenty generations, failed to show any differences on several different diets and so there was no evidence that adaptation to laboratory conditions was important.

10. The lack of evidence for co-adaptation apparently conflicts with what has been claimed for other species. Such differences are discussed.

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