EFFECT OF ENVIRONMENT UPON THE MANIFESTATION OF HETEROSIS AND HOMEOSTASIS IN *DROSOPHILA PSEUDOOBSCURA*

M. O. VETUKHIV¹ AND J. A. BEARDMORE²

Department of Zoology, Columbia University in the City of New York

Received November 3, 1958

A NUMBER of studies involving the comparison of the F_1 and F_2 generations from crosses between geographic populations of *Drosophila* with the populations themselves have been carried out. In general the findings were that F_1 hybrids showed a heterotic increase in Darwinian fitness over the parental populations and the F_2 generation showed a breakdown of heterosis.

Possible explanations for this kind of effect are of some interest and have been discussed by several workers, for example, by WALLACE and VETUKHIV (1955). However, the present experiments were initiated with another end in view. A number of observations had shown that the relative performance of two or more genotypes may be different under different environmental conditions. It appeared interesting to test whether the heterosis and breakdown observed in crosses between geographic populations at 25°C was maintained over a range of environmental conditions. Experiments of BEARDMORE (unpublished data) had shown that the superiority in developmental stability of F_1 hybrids over their inbred parents was dependent upon the type of environment in which the experiments were carried out. In an environment embodying a regularly fluctuating temperature cycle, *Drosophila* F_1 hybrids were not significantly more stable or homeostatic than the inbred parental lines, although under constant temperature conditions the F_1 superiority was consistently manifested as a lower degree of asymmetry of the sternopleural chaetae.

In the present study we tested whether F_1 s derived from crosses between geographic populations show heterosis and whether the F_2 s show breakdown over a range of environmental conditions. Comparable experiments testing flies homozygous and heterozygous for various second chromosomes of *D. pseudoobscura* have been described by DOBZHANSKY and WALLACE (1953). These experiments were concerned essentially with the between-homologues component of heterosis in F_1 s, whereas in the present study we have looked at the over-all interaction between haploid sets of chromosomes in the F_1 and, as well, at recombination between sets in the F_2 generation.

MATERIALS AND METHODS

The material for the experiments consisted of 16 strains of *D. pseudoobscura* derived from wild flies collected at Gunnison, Colorado, and of ten strains from

¹ Deceased June 1959.

² Present address: Department of Genetics, University of Sheffield, England.

Mather, California. Each strain is descended from a single impregnated wild female. All the strains were homozygous for the Arrowhead gene arrangement in the third chromosomes. The strains were the same as those used in experiments reported previously by VETUKHIV (1953, 1956, 1957).

Virgin females were taken from one half of the strains and placed together with males from the other half of the strains. Reciprocal crosses were made using males from the first half of the strains and the females from the second half. Mather $\times$ Gunnison F_1 and F_2 hybrids were produced, and reciprocally, Gunnison $\times$ Mather F_1 and F_2 generations. The components of Darwinian fitness used to compare the three generations of flies were, larval viability (to eclosion), fecundity, (i.e., egg production) and the quantitative asymmetry of the wings. The larval viability was measured by putting 20 small first instar larvae within each of a number of vials for each genotype. The food used was KALMUS (1943) synthetic medium with a controlled amount of dried inactive yeast added.

For the fecundity studies, five females and five males were placed in each vial. Since five replicates were made, 25 females and 25 males were taken for each cross. Thus 50 females of the parental forms were taken for egg laying in each experiment, 50 females of F_1 s and 50 of F_2 s. Paper spoons with cream-of-wheat and molasses medium with a drop of freshly made suspension of Fleischmann's yeast were changed daily in each vial and the eggs were counted.

The wing asymmetry, that is the difference in length between right and left wings regardless of sign, was determined by detaching and measuring with an eyepiece micrometer both wings from each of 50 flies of each sex for each genotype.

The three environments used were, 25°C, the temperature at which the previous work had been carried out, 22°C, and a fluctuating temperature condition (designated F°) which changed from 17°C to 27°C and back each 24 hours.

The viability data

The data presented in histogram form in Figure 1 show for each of the six genotypes, the mean number of flies hatching per 20 larvae. The mean is derived from 15 replicate vials per genotype.

The data are split into three groups by environment. At 25° the F_1 s show significantly greater survival than the parental forms and the tendency to break down is observed in F_2 s against F_1 s. In comparison with the P population, F_2 occupies an intermediate position, it is greater than one of the parents and less than the other one. In the 22°C and F° environments no significant differences are observed between genotypes, with the exception that in F° , F_2 is apparently superior to F_1 .

The variability of survival of larvae (that is, the variance in sets of replicate vials) is shown in Table 1. It is readily seen that at 25°C, and only at that temperature, the parental genotypes show greater variance than the F_1 s, as do the F_2 s. This variance is in all cases of both genetic and environmental origin but the genetic variance will be expected to be equal in F_1 and in P generations; hence a

lower phenotypic variance is indicative of a superior homeostasis in the development of the F_1 phenotypes. In the F_2 , of course, the fact that the variance is significantly greater than in F_1 could mean that there has been a breakdown of buffering capacity, that is, an increased sensitivity to environmental disturbance and inequalities. We are unable, however, to distinguish between the two com-

FIGURE 1.—Mean number of flies hatching per 20 larvae. M denotes the flies from Mather, California. G denotes the flies from Gunnison, Colorado. The horizontal line across two bars shows the mean between the values presented by each of the bars.

TABLE 1

Comparative variation in survival of larvae in replicate cultures

VARIANCE RATIO	P/F_1		F_2/F_1		F_2/P	
	min	max	min	max	min	max
25°	2.7 ⁺	6.0 ⁺⁺	2.7 ⁺	5.0 ⁺⁺	0.6	1.9
22°	0.5	2.1	1.2	0.5	0.48	1.0
f°	0.6	0.7	0.65	0.78	0.95	1.2

min = smallest variance ratio of four possible.
 max = largest variance ratio of four possible.
⁺ $P = < 0.05$.
⁺⁺ $P = < 0.01$.
 f° = F_0 .

ponents of variance, genetic and environmental; and recombination and segregation should in F_2 give rise to a generation with a genetic variance greater than in F_1 and P generations.

Note that in both of the other environments no significant differences are evident.

The fecundity data

The fecundity data are seen in Figures 2 and 3. Figure 2 shows the mean lifetime egg production per female, Figure 3, the mean daily egg production. As with the viability figures, the data are divided according to the test environment.

To consider first the data in Figure 2, it is clear that the F_1 s are heterotic in terms of egg production at 25°C, and the F_2 generation is less fecund than P, though this latter difference is statistically not significant. In both of the other environments no statistically significant heterosis or breakdown was detected. This picture is exactly paralleled by that shown by Figure 3—the mean lifetime egg production— F_1 heterosis at 25°C, and no significant breakdown in F_2 ; at 22°C and F_1 17/27 no statistically significant heterosis or breakdown observed in any case.

FIGURE 2.—Mean daily egg production per female.

The asymmetry data

The experimental results are shown in Figure 4 which expresses the quantitative difference between the two wings of individuals as the mean difference in micrometer units. One such unit is equivalent to about 1/800 total distance between the base of a bristle just anterior to the humeral cross vein and the tip of the wing, this being the distance chosen to represent wing length.

None of the comparisons of pairs of genotypes within environments is significant. It is noteworthy, however, that the difference between F_1 and P generations at 25° is the largest difference between two such pairs of observations and we have some grounds for feeling that a heterotic effect is present, although we cannot detect it. There are two reasons for this view. The first is that the test of significance utilized here is the analysis of variance and the error variance in all cases proved to be extremely large; hence our sample size of 100 individuals per genotype was probably too small and could well have been tripled. The second point is that the difference between the two wings of an individual fly is dependent to some extent upon the absolute length of the wings involved; the larger the wings, the greater the difference between wings irrespective of buffering ability.

FIGURE 3.—Mean life egg production per female

FIGURE 4.—Mean wing asymmetry per fly (in micrometer units).

Now the flies produced at 25°C were, of course, appreciably smaller than those produced in the other two conditions and this means that one unit of asymmetry at 25°C represents less efficient stabilization of development than does one unit of asymmetry at 22°C. It follows that the observed difference between F₁ and P at 25°C is indicative of a greater difference between homeostatic properties of the two generations than appears at first sight.

DISCUSSION

The data are summarized in Table 2. The + and - signs indicate the direction of difference in the comparisons P-F₁, F₁-F₂, P-F₂ and the figures are the probabilities for the various comparisons.

The results are interesting for several reasons. In the first place, they show that heterosis when it appears in F₁ hybrids is not necessarily found in all characters studied. The mean longevity of the flies is shown in Figure 5. It is apparent that there is no heterosis for this character in any environment. This is in contrast to findings of VETUKHIV (1957) whose work showed that F₁s between geographic populations exhibited the heterosis and F₂s showed the breakdown. This difference in experimental findings may not be very meaningful but it is possible that the difference is due to the fact that in the present study, the measurements of longevity were made on flies laying eggs more prolifically than in the original study, when the measurements were made upon flies kept under conditions not optimal for egg laying. The inference is that the energy in the one case is directed in the channel of egg production and in the other case into maintaining the anti-senility defenses of the body and thereby promoting longevity.

We bring up this point for it does seem to us that here we may have alternative channeling of biological effort, the direction of which is dependent upon the immediate environment of the flies and not just a case of heterosis being shown in

TABLE 2

Significance level of differences between genotypes

	Viability	Eggs Per Day	Eggs Per Life	Fly Days	Wing Asymmetry	
$F_1 - P$	+ 0.01	+ < 0.05	+ < 0.05	+ > 0.20	- > 0.20	25°
$P - F_2$	- > 0.20	+ > 0.20	+ > 0.05	- > 0.20	+ > 0.20	
$F_1 - F_2$	+ > 0.20	+ < 0.05	+ < 0.01	- > 0.20	- > 0.20	
$F_1 - P$	+ > 0.20	+ > 0.05	+ > 0.20	- > 0.20	o > 0.20	22°
$P - F_2$	- > 0.20	+ > 0.20	- > 0.20	- > 0.20	+ > 0.20	
$F_1 - F_2$	+ > 0.20	+ > 0.05	+ > 0.20	- > 0.20	+ 0.20	
$F_1 - P$	- > 0.20	+ > 0.20	+ > 0.20	- > 0.05	- > 0.20	f° 17-27
$P - F_2$	- > 0.05	+ > 0.05	+ > 0.05	+ > 0.20	+ > 0.20	
$F_1 - F_2$	- < 0.05	+ > 0.05	+ > 0.20	- > 0.20	+ > 0.20	

f° = F°.

same characters (viability and fecundity at 25°C) and not in others, e.g., longevity. Such situations have been noted by a number of workers, e.g., MORIWAKI *et al.* (1956), LEVINE (1952).

The second and more important point is that in this study it is quite clear that while in general the results obtained at 25°C agree with those utilizing geographic populations carried out previously, the same cannot be said of the 22° and F° (17/27) data. In the two latter environments we have no evidence for the occurrence of heterosis in crosses between the geographic populations used. For this reason we have presented no data on what DOBZHANSKY (1953) has called macroenvironmental variance. There is little doubt that if we did, we would not find the kind of result obtained by DOBZHANSKY and WALLACE (1953), i.e., F_1 s (in their cases, chromosome heterozygotes) showing less macroenvironmental variance than homozygous parents.

We are led to consider that the lack of concordance between the different environments is simply a reflection of the relative stringencies of the environments used. A more stringent environment is closer to the biological limit for one or more factors, whether this be the thermal death point, oxygen tension, food supply, or any other. Now, as has been demonstrated by a number of workers and emphasized notably by LERNER (1954), euheterosis is connected with devel-

FIGURE 5.—Mean fly days.

opmental homeostasis, therefore, it seems logical to expect to find heterosis most readily manifested under stringent or otherwise unfavorable conditions. Of course, this does not imply that heterosis will not be seen under more nearly optimal conditions. There is some evidence to support this line of reasoning. Here we may mention the work of MORIWAKI *et al.* (1956) who investigated the comparative viability of gene-arrangement homozygotes and heterozygotes in *D. ananassae*. In two components of viability (prepupal and postpupal), both biologically significant indices of heterosis, no heterosis was observed under optimal conditions but was observed, "at least in part" under unfavorable or stringent condition.

We feel that it is legitimate to suppose that for *D. pseudoobscura* 25° C is a more stringent environment than is 22° C or the fluctuating condition 17° ⇌ 27° C. It produced the lowest mean viability of all genotypes taken together and the flies were less fecund. This means that heterosis is more likely to be manifested in this environment than in the others. It would be possible to alter some other components of the environment to produce stringency even in 22° C and „F”° conditions. If this were done we should then expect to find heterosis and possible

F₂ breakdown. The idea that the species in general is adapted to a temperature range in which 22° C is more optimal than 25° C has to be tested critically but is ostensibly sound. It is possible, however, that the populations used in these experiments possessed a special adaptation to low temperatures, for the strains of which they are composed have been maintained in the laboratory for many years at 15° C. A shift to 22° C is clearly less than a move to 25° C and this would mean that 25° C environment would in any case be more difficult or stringent than the other two.

The fundamental problem, however, why F₁ heterosis and F₂ breakdown are so often observed in experiments of the type described is still unsolved. The discussion by WALLACE and VETUKHIV (1955) postulated that interpopulational F₁ hybrids are superior to the parental populations by virtue not of heterozygosity alone, but also due to coadaptation of the entire genetic systems. To quote WALLACE and VETUKHIV (1955), "This heterosis must be ascribed to heterozygosity for different but integrated gene systems." Some components of coadaptation are involved here, however; notably those concerned, it appears, with the interaction of loci within a single chromosome, and perhaps, less importantly, those concerned with the relationship of different chromosomes in a haploid set. If a breakdown in F₂ is the usual result, then these components must be most affected, and the disruption of the coadapted system leads to a F₂ inferiority. On the other hand, what MATHER (1943) has called relational balance seems less important in our material.

SUMMARY

Some components of Darwinian fitness, i.e., larval viability, fecundity and asymmetry of the wings, have been studied in P, F₁ and F₂ of crosses between geographic populations of *D. pseudoobscura*. The three environments used were: 25° C, 22° C and a fluctuating temperature 17° to 27° C and back each 24 hours. The F₁ hybrids generally showed heterosis in their viability, fecundity, and in wing asymmetry at 25°, but not at 22° or under fluctuating temperatures. F₂ hybrid breakdown occurred in the same traits at 25°, but not in the other environments.

The results obtained suggest that heterosis may be manifested more readily under stringent than under optimal environmental conditions. Among the environments created in our experiments, the temperature of 25° C is presumed to be farther removed from the optimum for the flies than 22° or the fluctuating temperature cycle.

ACKNOWLEDGEMENT

Thanks are due to PROFESSOR TH. DOBZHANSKY for his interest and advice. The hospitality enjoyed by J. A. BEARDMORE during the tenure of a Commonwealth Fund Fellowship in PROFESSOR DOBZHANSKY's laboratory is also gratefully acknowledged.

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