GENOTYPIC-ENVIRONMENTAL INTERACTIONS FOR VARIOUS TEMPERATURES IN DROSOPHILA MELANOGASTER

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INTERACTIONS between genotype and environment are defined as the differing behavior of similar genotypes in diverse environments. Evidence is accumulating that such interactions are common (LERNER 1958; MATHER 1955.) The mechanism by which populations react to different environments is difficult to assess; however, by definition, populations of genotypes exhibiting greater stability or homeostasis than others are less liable to be affected by fortuitous fluctuations of the environment. Populations exhibiting a high degree of stability or homeostasis may be expected, therefore, to show genotype-environmental interactions of less importance than populations with low stability.

In a population with low stability, the genotypic variance obtained in one environment may be radically different from that obtained in another, whereas in a population with high stability there should be a greater correlation between various environments. A critical factor in a breeding program is the magnitude of the genotypic variance of a population over the multiplicity of environments likely to be encountered.

In this paper, data are presented on the ability of *D. melanogaster* larvae to emerge as adults under six different temperature regimes. Three inbred lines, their hybrids and the F_2 generation are considered in an attempt to study the relationships between homozygosity, homeostasis or stability, and the magnitude of genotype-environmental interactions. In a similar, but considerably smaller experiment, THODAY (1953) studied the mean number of adults emerging from cultures of ten eggs for two inbred lines and their hybrids under four temperature regimes. He noted that the F_1 flies survived extreme temperatures better than the parents and therefore had greater stability for the specific environments involved. He did not, however, carry his analysis further than this.

MATERIALS AND METHOD

The three inbred lines were:

Oregon (OR)—sib-mated for 260 generations.
Sacramento (SAC)—sib-mated for 172 generations.
Bikini (BIK)—mass inbred since 1947.

The six possible F_1 hybrids and F_2's are included in the analysis.

Before commencing the main experiment, hatchability estimates were made from 20 samples of 150 eggs for each inbred line, F_1 and F_2. From this study, the

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number of eggs expected to give 150 larvae was calculated, hence providing a tolerably accurate method of ensuring approximately 150 larvae per replicate in the main experiment. Each replicate in the main experiment consisted of a vial with about ten gms of food in which the number of eggs expected to produce 150 larvae was placed. Ten replicates of each inbred line, F_1, and F_2 were set up for each of the following six temperature regimes:

15°C
24°C for eight hours and 15°C for 16 hours (24°/15°C)
24°C
24°C for eight hours and 31°C for 16 hours (24°/31°C)
31°C
31°C for 16 hours and 15°C for eight hours (15°/31°C).

The flies at 15°C were kept in a refrigerator, those at 24° in a constant temperature room and those at 31°C in a waterbath. At 10 A.M. and 6 P.M., the replicates under the fluctuating temperatures were changed from one temperature to the other, so that the 16 hour period represents the night and the eight hour period the day.

At 15°C flies take 20 days to emerge on the average, compared with ten days at 24°C, and eight to nine days at 31°C. The temperature 31°C was chosen as it is partly or wholly lethal to some strains.

The adult flies were scored every two days until emergence was complete. The number of adult flies emerging from 150 larvae provides a measure of the combined effects of larval and pupal mortality. In THODAY's (1953) experiment, no correction for differential hatchability was made. His emergence figures therefore depended on hatchability as well as larval and pupal mortality.

Hatchability estimates: The hatchability estimates are presented in Table 1. The hatchability of the OR and SAC inbred lines is low compared with BIK. The two techniques of inbreeding, sib-mating and mass inbreeding, may explain this difference. Under sib-mating, randomly selected single pair matings form each generation, whereas under mass inbreeding, flies are transferred in mass from generation to generation, so that competition is more severe and favorable genotypes, which are probably heterozygotes, may unconsciously be selected more readily than under sib-mating. The F_1 figures are, with minor exceptions, similar

TABLE 1

Hatchability estimates—mean number of hatched eggs from 20 replicates of 150 eggs with standard errors

OR	134.3 ± 4.5	OR × SAC	127.6 ± 5.9	OR × BIK	141.7 ± 3.6
SAC	119.8 ± 4.5	SAC × OR	117.6 ± 7.6	BIK × SAC	142.2 ± 3.2
BIK	144.7 ± 2.8	BIK × OR	141.1 ± 3.3	SAC × BIK	132.8 ± 7.0
			F_2's		
	OR × SAC	147.7 ± 2.4	OR × BIK	147.8 ± 1.1	
	SAC × OR	146.8 ± 2.2	BIK × SAC	139.3 ± 3.7	
	BIK × OR	147.1 ± 2.0	SAC × BIK	147.1 ± 2.5	

in magnitude to the inbred lines. The crosses with BIK as the maternal parent give values similar to the BIK inbred line.

The maternal parent appears to determine hatchability of the eggs, hence the F_1's and inbred lines hatched from eggs of the same maternal genotype give similar hatchability estimates. A similar result was obtained by CLOUGH and COCK (1957) in chickens.

Similarly, the standard errors of the hatchability estimates are equivalent in magnitude between the inbred lines and F_1's. The standard error of the BIK inbred line and the crosses with BIK as the maternal parent are smaller, on the whole, than the standard errors of the other inbred lines and crosses. This agrees with the interpretation that the BIK inbred line is perhaps more heterozygous than the SAC and OR inbred lines. The lesser variation of the BIK inbred line is an expression of its stability. The postulated heterozygosity of the BIK inbred line therefore gives it a superior homeostatic function compared with the SAC or OR inbred lines.

The F_2 hatchability figures represent the ability of the F_1 eggs to hatch. These hatchability figures, with the exception of the mating BIK × SAC, are higher than those for the corresponding inbred lines and F_1's. On the whole, therefore, these hatchability figures demonstrate heterosis. Furthermore, the F_2's have lower standard errors than the inbred lines and F_1's, thus exhibiting a superior homeostatic function compared with the F_1's and inbred lines. As hatchability appears to depend on maternal parent, these figures are therefore in agreement with recent theories on the relationship of heterozygosity and homeostasis.

Emergence of adults: In Table 2 the results for the emergence of adult flies are presented. Each entry represents the mean of ten replicates with its standard error. Marginal means are given for each temperature, inbred line, F_1, and F_2 with their standard errors. A few observations may be made directly on this table without further calculation.

At 24°C, the temperature at which the stocks were kept prior to the experiment, the highest proportion of larvae became adults. The next most favorable temperatures were 15°C and 24°/15°C. It is perhaps somewhat surprising that there is so little difference between the results for 15°C and 24°/15°C as a period at 24°C might have been expected to increase the emergence. Frequencies of emergence of adults from cultures kept at the three temperatures which included 31°C were not as high as the frequencies observed with cultures kept at the other temperatures. As might be expected, the lowest emergence was obtained at 31°C. At this temperature, the inbred lines OR and SAC had very poor emergence (an average of eight and 17 respectively). A period of eight hours daily at 24°C or 15°C and the remaining 16 hours at 31°C, was sufficient to increase the OR and SAC emergence two to eight times when compared with continuous exposure to 31°C.

The inbred lines OR and SAC generally appear to be very variable in response to different temperatures. The mass inbred BIK line is much more stable as shown by the average results for all temperatures. Furthermore, combining all

TABLE 2

Mean number of adults reared from 150 larvae at various temperatures from three inbred lines, their F_1's and F_2's with appropriate standard errors (see text)

Inbreds	15°C	24°/15°C*	24°C	24°/31°C	31°C	15°/31°C	Mean
OR	65 ± 15	90 ± 26	96 ± 19	58 ± 18	8 ± 4	69 ± 15	65 ± 33
SAC	90 ± 17	92 ± 14	86 ± 24	49 ± 14	17 ± 8	41 ± 11	63 ± 33
BIK	124 ± 18	127 ± 16	135 ± 17	106 ± 20	111 ± 21	120 ± 11	120 ± 20
F_1's							
OR × SAC	80 ± 16	80 ± 9	107 ± 8	88 ± 10	87 ± 26	76 ± 19	86 ± 19
SAC × OR	94 ± 20	73 ± 18	98 ± 12	72 ± 15	52 ± 11	62 ± 10	75 ± 22
BIK × OR	134 ± 15	133 ± 13	130 ± 14	128 ± 17	123 ± 23	98 ± 20	124 ± 21
OR × BIK	132 ± 16	126 ± 12	135 ± 14	125 ± 10	130 ± 12	104 ± 11	125 ± 16
BIK × SAC	119 ± 15	111 ± 19	132 ± 14	109 ± 14	111 ± 20	96 ± 23	113 ± 21
SAC × BIK	72 ± 17	58 ± 16	75 ± 17	47 ± 8	38 ± 9	48 ± 9	56 ± 19
F_2's							
OR × SAC	113 ± 22	127 ± 10	133 ± 16	107 ± 22	84 ± 13	101 ± 13	111 ± 23
SAC × OR	119 ± 20	125 ± 16	135 ± 11	111 ± 10	101 ± 21	101 ± 13	115 ± 20
BIK × OR	120 ± 19	109 ± 14	142 ± 15	137 ± 17	121 ± 13	113 ± 18	124 ± 20
OR × BIK	130 ± 13	122 ± 19	132 ± 17	119 ± 19	124 ± 8	124 ± 17	125 ± 16
BIK × SAC	117 ± 24	121 ± 17	127 ± 21	102 ± 19	122 ± 24	90 ± 14	113 ± 23
SAC × BIK	128 ± 16	130 ± 15	146 ± 10	91 ± 37	70 ± 25	109 ± 20	112 ± 33
Mean for each temperature	109 ± 28	108 ± 28	120 ± 26	96 ± 33	86 ± 43	90 ± 29	

* This represents a fluctuating temperature. For eight hours of a day, larvae were exposed to 24°C and for 16 hours to 15°C. For the other two fluctuating temperatures, 24°/31°C and 15°/31°C, the first temperature represents the eight hour period, and the last the 16 hour period.

temperatures, almost twice as many flies emerge in BIK as in OR and SAC. Once again this is probably due to the difference between mass inbreeding and sib-mating for the inbreeding system. The relative stability of the BIK inbred line as compared with OR and SAC is obvious from the relative magnitudes of the standard errors of the marginal means. Hence, it may be postulated that the BIK inbred line is more homeostatic than the OR and SAC inbred lines.

As might be expected, the two F_1 hybrids between OR and SAC are less variable in response to the temperature regimes than are the parental inbred lines. There is little evidence for a maternal effect, although the SAC × OR emergence is somewhat poorer than for OR × SAC. Using the mean of the two reciprocal F_1's, the possible existence of heterosis may be tested (Table 3). At the most unfavorable temperature, 31°C, there is very strong positive heterosis. Two other cases of positive heterosis occur (24°/31°C and 24°C). At 24°/15°C there is a degree of negative heterosis. Generally, there is a greater difference between the F_1 mean and the mid parental mean at the more unfavorable temperatures. The between temperature variability of the F_1 is much smaller than that for the two inbred lines.

Turning now to the F_1's between OR and BIK, it is obvious that there is no maternal effect (Table 2). Slight heterosis is present in some crosses and BIK is

dominant in the others. The variance of the marginal means for these crosses corresponds to that for the F_1's between OR and SAC (Table 2).

There was a large maternal effect between the hybrids BIK × SAC and SAC × BIK. The emergence of the hybrid SAC × BIK was much lower than BIK × SAC. For BIK × SAC there was, on the whole, no heterosis, and for SAC × BIK negative heterosis. As expected, the variances of these F_1's are low.

The F_2 crosses all produced similar numbers of adult flies. The maternal effect observed between BIK and SAC in the F_1 was eliminated, and the F_2's between OR and SAC gave more offspring than the F_1 hybrids. The variance components are, if anything, a little greater than the variances of the F_1's indicating the effect of F_2 segregation.

In Table 4, the analysis of variance of the data presented in Table 2 is given.

TABLE 3

Heterosis in the F_1 between OR and SAC

Temperature	Mean of reciprocal F_1's	Comment
15°C	87	SAC partially dominant
24°/15°C	76.5	negative heterosis
24°C	102.5	positive heterosis
24°/31°C	80	positive heterosis
31°C	69.5	positive heterosis
15°/31°C	69	OR completely dominant

TABLE 4

Analysis of variance of results in Table 2

	M.S.	F*	d.f.
Inbreds			
Genotypes	65,156	226.0	2
Temperatures	15,800	54.8	5
Genotypes × temperatures	2,492	8.6	10
Error	288		162
F_1's			
Genotypes	48,614	202.3	5
Temperatures	7,782	32.4	5
Genotypes × temperatures	697	2.9	25
Error	240		324
F_2's			
Genotypes	2,350	7.2	5
Temperatures	8,662	26.4	5
Genotypes × temperatures	1,619	4.9	25
Error	329		324
Interaction between inbreds, F_1's and F_2's	78,343		2
Total			899

* All the F values are significant at $P<0.001$.

The analysis is in three sections, inbred lines, F_1's and F_2's. Within each section there is a large temperature effect. A large genotype effect is present in the inbred lines and F_1's, but is smaller in the F_2 generation, as is obvious from the approximate equality of the numbers in the F_2 crosses (Table 2).

The relative magnitude of the mean squares for the three genotype × temperature interactions is of interest. The interaction is greatest for the inbred lines and smallest for the F_1's, the difference being significant ($P<0.05$). This shows that, as predicted from Table 2, the F_1's are more stable in response to the different environments than the inbreds. Thus, once again, it is shown that the F_1's show a higher degree of homeostasis than the inbreds. The F_2's have a genotype × temperature interaction intermediate in magnitude between the F_1's and inbred lines, as might intuitively be expected because of segregation in the F_2 generation.

Finally, there are two degrees of freedom which test the interaction between the inbred lines, F_1, and F_2 generations which is, as expected, highly significant when compared with the three error mean squares by means of the F test. This merely shows that the mean emergence of the inbred lines, F_1's and F_2's differs.

DISCUSSION

Basically, in this study, the adaptation of various inbred lines, their F_1 hybrids and F_2's, to a multiplicity of artificial environments was being investigated. Generally it was found that the heterozygous F_1 generation is less variable than the inbred parents. The stability of the F_1 generation was probably due to the superior homeostatic ability conferred upon it as a result of its heterozygosity. Evidence of this type has been presented by many authors among whom may be cited CLARINGBOLD and BIGGERS (1955), DOBZHANSKY and WALLACE (1953), LERNER (1954), ROBERTSON and REEVE (1955) and YOON (1955).

LERNER (1954), LEWONTIN (1956) and THODAY (1953) and others have stressed that characters of adaptive significance should be used in measuring homeostasis. Stability of fitness over all environments is the criterion of homeostasis. It is often difficult, for example, to see the relationship between some morphological characters and adaptive significance. LEWONTIN (1956) demonstrated that there is no simple relation between morphological uniformity and homeostasis for abdominal bristle number. Hence a population which exhibits a high degree of homeostasis is one in which characters of adaptive significance are kept constant under variable environmental conditions. However, the variability of other characters not directly related to fitness may be increased, as has been demonstrated for eye pigmentation in *Ephestia kühniella* by CASPARI and GOTTLIEB (1959). The ability of larvae to emerge as adults is obviously related to fitness and hence inferences on the relative variability of heterozygotes and homozygotes as representing different degrees of homeostasis ought to be tolerably valid.

The superior homeostatic ability of the F_1 hybrids is shown to lead, as expected, to a relatively small genotype × environment interaction when compared with the inbred lines. The F_2 generation occupies a position between the F_1's and in-

bred lines. A few environments will therefore give an estimate of the over-all behavior of the F_1, but many more environments will be needed for inbred lines. The widespread use of inbred lines suffers from the defect of considerable variation between environments. CLARINGBOLD and BIGGERS (1955) and MCLAREN and MICHIE (1956) have pointed this out for inbred lines of mice.

Many genotype-environmental interactions have been reported in the literature, and the problem has been discussed theoretically (COMSTOCK 1955; DEMPSTER 1955; LERNER 1958; and MATHER 1955). It is of great interest to both the evolutionist and the breeder to estimate the magnitude of the genotype-environmental interactions resulting from subtle, but nevertheless important micro-environmental changes. PARSONS and ALLARD (in press) present data on seed size in the lima bean (*Phaseolus lunatus* L.) grown in an apparently constant environment over a period of six to eight years demonstrating significant interactions between genotype and environment. All these studies indicate the need to study populations in many environments if predictions of possible progress under selection are to be reliable. The data reported in this paper emphasize the differential behavior of inbred and noninbred individuals in different environments and show that predictions made on heterozygous individuals exhibiting homeostasis would be more reliable than nonhomeostatic inbreds.

Finally, these data bring out an interesting contrast between mass inbreeding and inbreeding by sib-mating. The mass inbred BIK strain is shown to be almost equivalent in homeostatic ability to the F_1 hybrids. This shows up at extreme temperatures such as 31°C when BIK emergence is much higher than the OR and SAC emergence. Thus the mass inbred BIK line is better buffered against environmental extremes than the OR and SAC inbred lines. It is unlikely that this is due to the environments to which these inbred lines were subjected before bringing them into the laboratory, as all three inbred lines have been under constant temperature conditions for approximately ten years. The more likely interpretation is that mass inbreeding permits the unconscious selection of heterozygotes more readily than under sib mating. The hatchability figures confirm this interpretation, for these were all obtained at 24°C, so that the original environment of the BIK strain can have little or no effect.

In chickens, SCHULTZ (1953) found that the inbred lines were more sensitive, and the crosses between them less sensitive to environmental changes than the more or less randomly bred birds of production flocks. Similarly MCLAREN and MICHIE (1956) found that randomly bred Mousery mice were almost as uniform as the F_1 hybrids and the inbred lines more variable than the Mousery mice. In the randomly bred chickens and Mousery mice there is probably an unconscious selection of fitter heterozygotes as has been suggested for the mass inbred BIK line.

SUMMARY

1. Three inbred lines of *Drosophila melanogaster* were crossed in all combinations and the F_2 generation grown. Hatchability estimates were made, and the

ability of 150 larvae to emerge as adults under six different temperature regimes studied.

2. The hatchability of the eggs from the F_1 flies exhibited heterosis and decreased variability compared with the inbred lines. Thus homeostasis, superior to the inbred lines, is indicated.

3. The inbred lines were more variable than the F_1 hybrids in response to temperature treatment as measured by the emergence of 150 larvae as adults. Furthermore, the inbred lines exhibited a larger genotype-environmental interaction than the hybrids. The hybrids therefore have a better homeostatic mechanism than the inbred lines.

4. Two of the inbred lines (OR and SAC) were inbred by sib mating, and the third (BIK), by mass inbreeding. The BIK inbred line was almost equivalent to the hybrids in homeostatic ability, probably because of the greater opportunity for the unconscious selection of favorable heterozygotes.

5. Some of the implications of the results are discussed.

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