DEVELOPMENTAL STABILITY IN CONSTANT AND FLUCTUATING TEMPERATURES

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1. INTRODUCTION

THE comparative study of different genotypes has to be carried out in controlled environments, and for convenience, such conditions are usually made as constant as possible. Under natural conditions, however, many biological populations are exposed to variations both in time and space and must be adapted to survive such variations. Information as to the effects of fluctuating environments on individuals and populations is clearly desirable.

Regular fluctuations with periods greater than generation time may provoke characteristic cyclic changes in the gene pools of populations (Dobzhansky, 1950; Dubinin and Tiniakov, 1945; Timoféeff-Ressovsky, 1940). On the other hand, both regular and irregular fluctuations of short period must be met by the individual's developmental homeostasis (Lerner, 1954), developmental stability (Mather, 1953) or developmental flexibility (Thoday, 1953) and it might therefore be expected that the genotypes best adapted to constant conditions would be genetically different from those best adapted to fluctuating conditions. Very few informative comparisons have been made in this connection but suggestive results have been obtained by Tebb and Thoday (1954*a*, *b*). It therefore seemed worth while to compare the developmental stability or homeostasis of different *Drosophila* genotypes in constant and fluctuating temperature conditions.

2. MATERIALS AND METHODS

(i) *The environments*

Tebb and Thoday, 1954*a*, *b*, obtained a diurnal fluctuation of temperature by moving their cultures from one constant temperature room to another each morning and evening. Apart from the labour, this system has the disadvantage that the precise mean temperature is not easily controlled. An incubator which produced a fluctuating temperature that would be relatively precisely controlled was therefore designed and built.

This incubator provided an environment, which will be termed F20°/30°, with a mean temperature of 25° C. The temperature at midday was 30° C. and that at midnight 20° C. Fig. 1 shows a typical recording from a thermograph in the incubator.

The fluctuation was produced by a clock that revolved once each 24 hours. The clock was connected to the thermostat control by a crank system so that the revolution of the clock caused oscillations of the thermostat spindle and hence diurnal fluctuation of temperature. In practice this mechanism worked very well although theoretically the type described by Howe (1956) is probably preferable.

For most of the work (but see below, ii) the constant temperature environment was provided by a water-jacketed incubator maintained at 25° C. Humidity in both incubators was maintained in the range 35-50 per cent. R.H. and the observed diurnal oscillation of relative humidity in F20°/30° rarely exceeded ±4 per cent. in the air surrounding the cultures. Within the culture bottles it would presumably be even less.

(ii) *Stocks of* Drosophila melanogaster

In the first group of experiments, cultures of the highly inbred stocks Oregon and Samarkand and of their crosses were raised in the constant and in the fluctuating environments. Each culture was derived from a double pair mating. Five replicates per genotype were grown in each experiment. There were eight nominally replicate experiments, but these differed slightly. For experiments 1-4 a culture room ostensibly maintained at 25° C. but with appreciable variations in temperature was

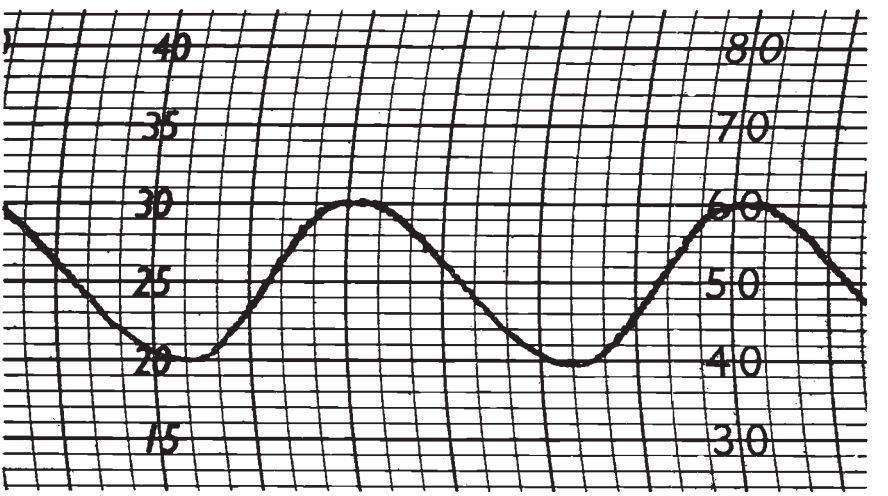

FIG. 1.—Thermograph recording taken in the fluctuating temperature incubator.

used to provide the 25° C. environment. An accurately controlled incubator maintained at 25° C. was available for the later experiments.

In the second group of experiments two " wild " stocks were used, each descended from an inseminated female, one taken at Bayfordbury and one at Sheffield (Bannerdale). Five replicate cultures of each of the two stocks and the two reciprocal crosses were grown in each experiment, each culture being derived from a single pair of parents.

(iii) *The index of homeostasis*

The index of homeostasis in the present study was essentially that used by Mather (1953), Tebb and Thoday (1954*a*) and Thoday (1958), asymmetry of sternopleural chaeta number. Despite criticisms (*e.g.* Waddington, 1958) there now seems to be little doubt that this character is a useful, if limited, index of developmental homeostasis. The results of Mather (1953), Tebb and Thoday (1954*a*), Beardmore (1956) (quoted in Thoday, 1958), Beardmore, Dobzhansky and Pavlovsky (1960), are consistent in indicating some negative correlation between asymmetry and Darwinian fitness. A discussion of the value of this character will be found in Thoday (1958).

3. RESULTS

(i) *The inbred stocks and their F_1's*

Table 1 shows the mean asymmetry per 20 flies of sternopleural chaeta number for the two inbred lines, O and S, and for the two reciprocal F_1's in the 25° C. and F20°/30° environments. These mean values are based on the total data from the eight experiments. In the analysis of variance of the asymmetry data (table 2) only the data

TABLE 1

Asymmetry of sternopleural bristle number in O, S and their F_1's in two environments (each entry represents the summed asymmetry of 20 flies averaged over 80 cultures with the sexes combined)

Environment	Genotype					
	O	S	Mean P	O×S	S×O	Mean F_1
Constant 25° C. .	21·22	17·52	19·36	18·12	18·32	18·22
Fluctuating 20°/30° .	20·20	18·44	19·22	20·20	19·66	19·92

from the last four of these experiments have been analysed, for only in these experiments was the temperature properly controlled.

The first item in the analysis of variance set out in table 2 is very highly significant and indicates differences between genotypes. This when partitioned proves to be largely the result of difference in mean

TABLE 2

Analysis of variance of asymmetry in two inbred lines and their F_1's in two environments

Item	d.f.	Mean square	P
Genotypes	3	89·40	<0·001
Environments	1	47·30	>0·05
Environments × Genotypes . .	3	54·89	<0·01
(Env. × PF_1	1	149·9	<0·001)
Environment × Sex . . .	1	79·00	<0·02
Error	258	13·29	

asymmetry between the O and S parents, the O stock being characterised by a higher asymmetry than the S stock. This is quite possibly a result of a difference in sternopleural bristle number, T, rather than developmental stability, for in general, though not always (Mather, 1953), flies with more bristles are also more asymmetrical. Table 3 shows the mean T for hybrid and inbred genotypes in both environments and the value of T for O is appreciably greater than that for S.

A significant interaction between genotypes and environment is shown in the analysis (table 2). Of the total sum of squares for three degrees of freedom, by far the greatest part is absorbed by the one degree of freedom available for comparing the combined parents with the combined F_1's in the two environments. This $PF_1 \times$ environment interaction is significant as well below the 0·001 level of probability.

TABLE 3

Mean sternopleural bristle number (T) in O, S and their F_1's in two environments (sexes combined, each entry is the mean of 1600 flies)

Environment	Genotype					
	O	S	Mean P	O×S	S×O	Mean F_1
Constant 25° C. .	19·33	16·83	18·08	18·24	17·59	17·91
Fluctuating 20°/30° .	19·32	16·69	18·07	18·04	17·23	17·63

If the data are divided and separate analyses carried out for each environment, it is found that the F_1's are significantly less asymmetrical than the parental types in 25° C. but not in F20°/30°. The interaction in the analysis of the total is therefore a result of this relative difference of behaviour of the inbreds and F_1's in the two environments. The $PF_1 \times$ environment interaction cannot plausibly be attributed to simple

TABLE 4

Mean number of flies hatching per 10 eggs in O, S and their F_1's in two environments (each entry is the mean of 40 cultures)

Environment	Genotype					
	O	S	Mean P	O×S	S×O	Mean F_1
Constant 25° C. .	5·60	5·40	5·50	8·00	6·22	7·11
Fluctuating 20°/30° .	5·54	5·57	5·56	7·25	6·22	6·73

changes in chaeta number T. Although as shown in table 3, T is in general slightly, but significantly lower in F20°/30° than 25° C., the observed differences in T cannot alone be responsible for the relative differences in asymmetry of hybrids and inbreds under constant and fluctuating conditions.

The egg-eclosion viability of the inbred lines and their F_1 hybrids in the two environments is given in table 4. The viability of the hybrids is greater than that of the parental types, and the difference between P and F_1 viability is statistically significant in both environments ($P = < 0{\cdot}001$).

(ii) *The wild stocks*

The first experiment with the wild stocks involved testing the developmental homeostasis of the descendants of two wild gravid females, two generations after the initial capture. Tests were made in both environments. Both the stocks and the two F_1 crosses between them

TABLE 5

Mean asymmetry (per 20 flies) of sternopleural bristle number in two wild strains and their F_1's

Experiment	Genotype	Environment	
		25°	F°
I	Bannerdale	16·6	14·8
	Bayfordbury	23·0	20·6
	Bannerdale × Bayfordbury	18·7	17·4
	Bayfordbury × Bannerdale	19·1	18·1
II	Bannerdale	17·7	18·5
	Bayfordbury	21·2	21·2
	Bannerdale × Bayfordbury	19·4	19·8
	Bayfordbury × Bannerdale	19·5	20·1

were tested and the results are shown in the upper part of table 5. All genotypes are characterised by *lower* asymmetry in the fluctuating environment than in the constant environment and this difference is statistically significant ($P = < 0{\cdot}05$).

TABLE 6

Analysis of variance of asymmetry in two wild strains and their F_1's in two experiments

Item	d.f.	Mean square	P
Experiments	1	51	$< 0{\cdot}02$
Environments	1	13	$> 0{\cdot}20$
Genotypes	3	142·3	$< 0{\cdot}001$
Experiments × Environments	1	44	$\simeq 0{\cdot}02$
Experiments × Genotypes	3	16	$> 0{\cdot}2$
Experiments × Sex	1	11	$> 0{\cdot}2$
Error (pooled)*	149	8·48	

* The error mean square is based upon a sum of squares which includes the variance between cultures and those items not listed in the table whose individual mean squares were similar to the original error mean square.

The second experiment with these stocks was carried out in the same way as the first, but ten months later. The stocks had been maintained in the intervening period in mass culture under normal laboratory conditions at 25° C. The mean asymmetry for the four genotypes in the two environments is shown in the lower half of table 5.

Though there is an obvious suggestion that flies cultured in the fluctuating environment are now *more* asymmetrical than those cultured at 25° C., in fact the difference is not significant, $P > 0{\cdot}05$.

An analysis of variance of the combined data from the two experiments is shown in table 6. There are significant items due to experiments, genotypes and the experiments × environments interaction. The genotypes item is a reflection of the fact that, as with the O and S stocks, the Bayfordbury and Bannerdale stocks have different mean asymmetries largely because they have different total bristle numbers. The F_1's have intermediate values of both asymmetry and T. The experiments × environment item arises because the mean asymmetry in F20°/30° was higher in the second experiment than in the first, the asymmetry values in 25° being more or less similar in the two experiments. It is this which is responsible for the experiments item.

The interaction may be interpreted as follows. In the first experiment all genotypes showed greater developmental stability in F20°/30° than in 25° C. but in the second experiment developmental stability was lower, though not significantly so, in the fluctuating than in the constant environment (see fig. 3).

4. DISCUSSION

(i) *The inbred stocks and their F_1's*

In the 25° C. environment, the developmental stability of the F_1's between the two inbred lines O and S, as measured by asymmetry of sternopleural chaetæ, is superior to that of the inbreds. This agrees with Mather's (1953) findings. In the fluctuating condition, however, the hybrids are at least as unstable in development as the inbred types (fig. 2). This is a somewhat surprising result for there is no obvious reason why the F_1 superiority should not be retained in the fluctuating environment. There are two hypotheses, not necessarily mutually exclusive, that might explain this result. The first hypothesis assumes that a change of environment may produce a change in dominance relations of the type postulated by Lewis (1953). Using his model any phenotypic character affected by given environmental changes would display a variance in F_1 hybrids which would be greater or less than that of the parents depending on whether the genes affected worked in the same direction as the environment or in the opposite direction. Applied to this experiment the model would require that during part or all of the high temperature half of the fluctuating temperature cycle (tending to reduce chaeta number), minus chaeta-number genes are on average dominant to their plus alleles in F_1 flies. Conversely, during the colder half (increasing chaeta number) plus genes would be dominant to minus genes. Such a response could well be adaptive, for there is evidence (Beardmore, 1956; see Thoday, 1958) that low chaeta number at high temperatures and high chaeta number at low temperatures may be adaptively useful. Now as chaeta number

determination seems to occur over a period of time (Plunkett, 1927) it is likely that a dominance change of the type postulated would have two consequences. These are an increased variance of F_1 chaeta number in F20°/30° and increased F_1 asymmetry in F20°/30°. The first of these consequences follows because unless the period of time during which chaeta number is determined is for all flies an exact multiple of the wavelength of the temperature oscillation, different flies will inevitably suffer different temperature experiences and produce

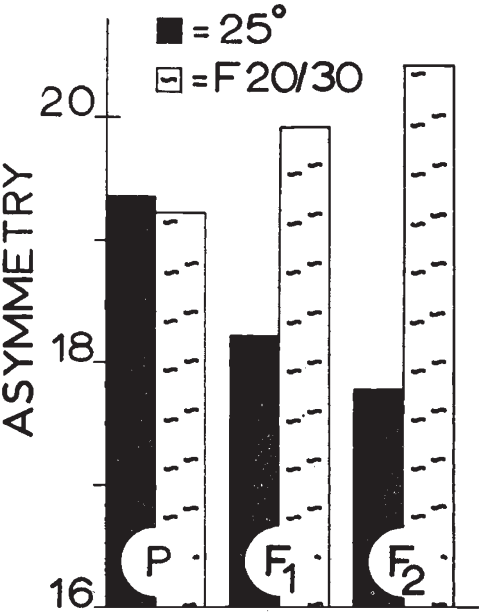

FIG. 2.—Mean sternopleural asymmetry per 20 flies of the inbred lines Oregon and Samarkand, their F_1's and F_2's in a constant and in a fluctuating environment.

different chaeta numbers. Similarly, unless chaeta production on the two sides of a fly were perfectly synchronous, an increase in asymmetry would result from the two sides developing at different temperatures. When the variance of chaeta number (T) in F20°/30° is examined it is found that (compared with 25° C.) it is increased in *both* F_1 and parental genotypes (table 7). This increase, assuming it results from the same cause in both P and F_1, cannot be due to a change in dominance. This evidence, therefore, does not support the dominance hypothesis unless it be supposed that the increase in variance is produced by different means in the inbreds and F_1's.

One further point requiring discussion is the difference observed between asymmetry and viability as indices of homeostasis in the two environments. The hybrids are more viable than the inbreds in both environments and clearly (table 4) there is no hint of a dominance change, though as viability can only vary adaptively in one direction, a dominance change would perhaps not be expected.

The alternative hypothesis is that, as the reversal of relative homeostasis is due primarily to the hybrids being absolutely less homeostatic in F20°/30° than in 25° C., the superiority of the F_1's in 25° C. arises not from heterosis alone but also because the F_1 combines genomes derived from parents both long adapted to constant 25° C. hence are themselves adapted to these conditions and when put together co-operate more successfully in 25° C. than in F20°/30°. Once more

TABLE 7

Coefficient of variation of sternopleural chaeta number in O, S and their F_1's in two environments

Genotype	Environment					
	25° C.			F20°/30°		
	♀	♂		♀	♂	
O . .	6·94	7·72		10·50	10·26	
S . .	7·44	7·42		10·99	10·60	
Mean P .			7·38			10·58
O×S .	6·91	7·45		10·38	11·22	
S×O .	6·82	6·87		10·75	10·79	
Mean F_1 .			7·00			10·79

the fact that the hybrid genotypes are more viable than the inbreds in both environments and the difference between the responses of asymmetry and viability must be considered. This difference may suggest that asymmetry cannot have any adaptive significance for the importance of viability as a component of Darwinian fitness is obvious. It is important to remember, however, that natural selection works upon the totality of the phenotype. Thus different phenotypic characters in an organism will always be at different distances from their respective optima depending both upon the relative adaptive importance of the character and upon the interactions between characters. In outbreeding species, natural selection should act in such a way as to favour the establishment of the maximum possible number of gene combinations which will produce the optimal phenotype. When different phenotypic characters are considered it will be clear that in general the number of such combinations is likely to be positively correlated with the relative importance of the character in tota Darwinian fitness. Thus it is likely to be very high for viability; as Wallace (1957) has shown, most increments of randomly produced

heterozygosity to a homozygous system improve viability. In the case of morphological characters the number of combinations is likely to be greater for genes mediating say symmetry in eye development than for those concerned with symmetry in sternopleural bristle development, for this may be presumed to be of a lower order in the scale of adaptive value than the former. Robertson (1955, 1959) discussing the relationship between fitness, genetic variance and various phenotypic characters in equilibrium populations arrived at a similar conclusion. This argument may be extended to include change of environment as well as change in genotype, the character of lesser adaptive significance being more (unfavourably) affected than the one of greater significance, when the environment changes. In the present study we may conclude that the loss of F_1 heterosis in terms of symmetry and its maintenance in terms of viability in F20°/30° are related to this.

The F_2 generation from crosses between O and S was shown by Mather (1953) to be less asymmetrical at 25° C. than the F_1's although less heterozygous. A small number of F_2 cultures grown in the present study produced data in agreement with this although the difference between F_1 and F_2 is not statistically significant (see fig. 2). More interesting is the fact that the F_2 flies in F20°/30° are ostensibly, though again not significantly, more asymmetrical than the F_1's. This may perhaps be taken to mean that not only are the F_2's in 25° nearer to an optimal state of heterozygosity than are the F_1's (as suggested by Thoday in Mather, 1953), but that the relational balance (Mather, 1943) in F_2 is specific or at least not general enough to give good homeostasis in F20°/30°. This may again be a consequence of the maintenance of the inbred parents in constant 25° C. for several hundred generations.

Although the argument advanced above should not perhaps be accepted without reserve the results appear to favour the second hypothesis rather than the first. They also provide another demonstration that heterozygosity as such does not inevitably lead to superior homeostasis although the validity of this statement depends upon the definition of homeostasis. A more important point is that these data emphasise the illegitimacy of extrapolating conclusions concerning fitness from one environment to another.

(ii) *Wild stocks*

The test with wild stocks showed that flies two generations removed from populations in the wild state showed a greater asymmetry in a constant temperature than in a fluctuating temperature. After approximately fifteen generations under normal laboratory conditions the populations were equally asymmetrical in both environments (fig. 3). This may be interpreted as meaning that the removal, for fifteen generations, of selection for adaptation to fluctuating conditions had resulted in some breakdown of the gene complexes established under such selection pressures in the wild. It would then, of course, be expected that adaptation to constant temperature conditions should

improve over the fifteen-generation period. Because of differences in T (and hence in asymmetry) between the two experiments it is difficult to be sure whether adaptation does so increase. The value of T showing the smallest between-experiment difference is that of the Bayfordbury stock and in this stock, adaptation to 25° C. as measured by asymmetry does improve between the two experiments. Though this difference is not statistically significant, the rather few data are ostensibly in agreement with the idea of adaptation and asymmetry being negatively correlated.

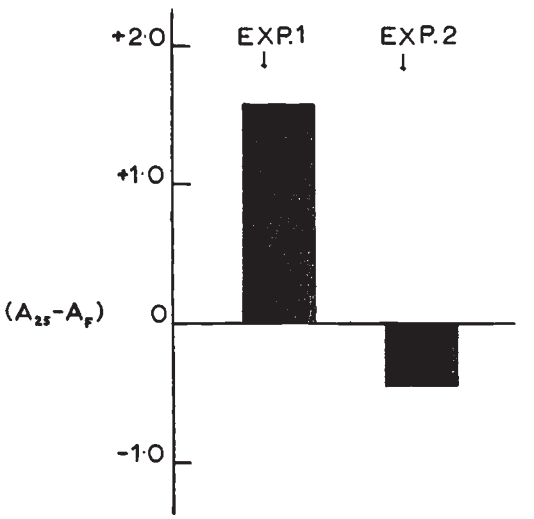

FIG. 3.—Mean difference per 20 flies of sternopleural asymmetry of " wild " strains in constant (A_{25}) and fluctuating (A_F) temperatures in two experiments.

Irrespective of the precise interpretation that may be placed upon these results it is clear that natural populations of *Drosophila* possess gene complexes capable of buffering development within a diurnally varying thermal environment and that such complexes are not necessarily capable of buffering development equally well under conditions of thermal stability*, to cope with which new gene complexes must be built up (*cf.* Bishop (1953) who observed 100 per cent. increase in the hatchability of eelworm cysts in fluctuating compared with constant temperature). The building-up of such genetic architecture seems to be essentially an adaptive process (Beardmore, 1956). These results, therefore, show that appreciable modifications of the gene pools of populations may occur when they are kept under laboratory conditions. The experiments with the inbred lines demonstrated that a difference between inbred and hybrid genotypes clearly shown at 25° C. disappeared under conditions of fluctuating temperature. Tests of wild chromosomes in constant laboratory conditions might in the same way give results which are not predictive of the attributes

* Note added in proof. Ford (in *Moths*, Collins 1955, p. 57) quotes the work of Kettlewell on body colour mutants of *Panaxia dominula* L. in which a similar situation may exist.

of such chromosomes in natural environments. Together these findings should engender caution in considering the relevance to wild populations of laboratory data.

5. SUMMARY

1. The developmental homeostasis of the inbred lines, Oregon and Samarkand of *Drosophila melanogaster* and of their F_1 hybrids, was studied in thermally constant (25° C.) and diurnally fluctuating environments (F20°/30°) of the same mean. Similar investigations on wild strains were also carried out.

2. In the constant temperature, homeostasis as measured by the mean asymmetry of the sternopleural chaetæ was greater in the F_1 hybrids than in the inbred lines Oregon and Samarkand, confirming Mather's (1953) results. The F_1's also showed heterosis in egg-adult viability. In the fluctuating environment the F_1's were no more homeostatic (symmetrical) than the inbreds but were significantly more viable. The fact that F_1's are more symmetrical than their inbred parents in constant 25° C. than in F20°/30° can be accounted for by (i) the dominance of genes affecting chaeta number varying in the fluctuating environment or (ii) better co-operation between the two halves of an F_1 genotype in constant than in fluctuating conditions consequent upon both halves being derived from parents themselves long adapted to constant 25° C. conditions.

The contributions of bilateral symmetry and egg-adult viability to total Darwinian fitness are of different order, the symmetry component being smaller (though not negligible). This difference is reflected in the number of genetic combinations giving the desirable phenotype for each character and in the response of particular genotypes in different environments.

3. Second generation descendants of two wild-captured inseminated females of different origin and crosses between these displayed less bilateral asymmetry under fluctuating temperature conditions than under constant temperature. After fifteen generations in the laboratory this difference was no longer evident in descendants of the wild flies. Hence wild populations are presumed to carry gene complexes buffering development under conditions of fluctuating temperature. Such genetic architecture is a product of natural selection and appears to be destroyed in populations maintained under constant temperature conditions.

4. Taken together these results indicate the necessity for caution in predicting what may happen in natural populations from laboratory data.

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