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# ACCLIMATIZATION TO HIGH TEMPERATURES IN INBRED AND OUTBRED *DROSOPHILA SUBOBSCURA*

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(With One Text-figure)

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## 1. Introduction

In recent years a number of workers have reported that $F_1$ populations between inbred lines often show greater phenotypic uniformity than do the parental populations. Haldane (1948) and Robertson & Reeve (1952) suggested that genetic heterozygosity might result in greater biochemical versatility in development, and hence in reduced sensitivity to environmental variations. Waddington (1942) coined the term 'canalization of development' for the processes which lead to constancy of adult phenotype; Lerner (1954) used 'developmental homoeostasis' in a similar sense. The word homoeostasis has the advantage of suggesting analogies with physiology, but canalization is better fitted to describe situations where alternative paths of development are open, for example, in sexual or in tissue differentiation.

The present investigation was designed to test a hypothesis concerning the nature of developmental homoeostasis, which was put forward by Maynard Smith, Clarke & Hollingsworth (1955); a similar hypothesis was put forward by Michie & McLaren (1955), and earlier by Thoday (1953). In our paper we pointed out that although in many cases fitness is conferred by developmental processes leading to constancy of adult phenotype, despite changes in conditions during development, there also exist circumstances in which high fitness requires a change in adult phenotype in response to changed conditions. In so far as outbred organisms possess greater powers of regulation during development, they would be expected to show in the former case greater constancy of phenotype, and in the latter a greater degree of adaptive modification. Thoday (1953) used the term 'developmental flexibility' to include both kinds of response: an unaltered phenotype in spite of environmental disturbances, and a changed phenotype adapting the organism to the conditions which evoked it.

Thus the hypothesis to be tested is as follows: organisms which show more efficient regulation during development, as expressed by greater constancy of adult phenotype when fitness requires such constancy, should also be capable of a greater change in phenotype in response to different conditions, when the changed phenotype increases the fitness of the individual.

Earlier work on *Drosophila subobscura*, reviewed by Maynard Smith *et al.* (1955), has shown that the decline in vigour with inbreeding is particularly rapid in this species, and that the $F_1$ hybrids between inbred lines differ from their parents in a high and relatively uniform rate of development, fertility and adult longevity. However, it is difficult in

*Drosophila* to find a morphological character of which it is possible to state *a priori* that particular changes will be adaptive in particular conditions. Therefore the character chosen for study was the capacity of adult flies to survive at high temperatures, and the changes in this capacity according to the temperature at which they had previously been kept.

It was known from previous work, particularly that of Mellanby (1939), that some insects are capable of acclimatization to different temperatures, although most work on this subject has been concerned with changes in the temperature of immobilization at low temperatures. However, striking changes have been found in the capacity of individuals of *D. subobscura* to withstand high temperatures, according to the temperature at which they have been kept, either as larvae or as adults. These changes can reasonably be regarded as adaptive; their extent has been compared in three inbred lines, and in the hybrids between them.

## 2. Material and methods

Three inbred lines, **B**, **K** and **NFS**, were chosen for study, since they are the only lines known to be structurally homozygous for all chromosomes. The **B** and **K** lines had been brother-sister mated for thirty-five, and the **NFS** line for twenty-five generations before the start of the investigation. $F_1$ hybrids between these lines are referred to as **K/B**, **NFS/K** and **NFS/B**, the female parent being written first in each case. The lines have been kept in half-pint milk bottles on a medium of maize meal, agar and molasses, with living yeast suspension added, at a temperature of approximately 20° C.

To obtain flies for experimental purposes, cultures were set up of three parents of each sex, using, for the inbred lines, sets of brothers and sisters. The parents were kept in mating vials for from 4 to 6 days at 20° C., and then transferred to culture bottles which were kept subsequently either at 15 or 25° C. In fact, the temperature in the former case fluctuated rather regularly between about 13 and 17° C., and in the latter between 24 and 25° C.

For each of the six genotypes, four groups of offspring were obtained, according to the temperature at which they had been raised:

(i) 15–15. Culture bottles containing parents transferred immediately to 15° C. Adults transferred to fresh food vials on the day of emergence, and kept at 15° C. until testing.

(ii) 15–25. As (i), but adult flies kept from emergence to testing at 25° C.

(iii) 25–15. Culture bottles containing parents transferred immediately to 25° C. Adults flies transferred to fresh food vials on the day of emergence, and kept at 15° C. until testing.

(iv) 25–25. As (iii), but adult flies kept from emergence to testing at 25° C.

The period from emergence to testing varied from 5 to 8 days at 15° C., and from 3 to 5 days at 25° C. Adult flies were transferred to a second fresh food vial on the day before testing.

Each group of flies will be referred to by two numbers, for example, 15–25, the first number referring to the temperature during egg, larval and pupal life, and the second to the temperature during adult life.

The survival times of individual flies were then recorded to the nearest 5 min. at a constant high temperature in dry air, a sample of six males and six females of each

genotype and group being tested. The temperature chosen was 33·5° C., since this gives survival times of the order of 100 min. An account of the apparatus used, and of the variations in survival time with temperature and with humidity, is in preparation. Errors due to differences in experimental procedure from one test to another were probably very small. The temperature was kept to within 0·1° C. of the required value throughout, and the air dried by passing through four columns of anhydrous calcium chloride. Repeat tests on flies of the same age, genotype and environmental history gave results which agreed very closely with one another.

## 3. RESULTS

The results of all tests are summarized in Table 1.

Table 1. *Mean survival times in minutes at* 33·5° C. *in dry air*

| Genotype... | B | | | NFS | | | K | | | NFS/B | | | K/B | | | NFS/K | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ♂♂ | ♀♀ | Mean | ♂♂ | ♀♀ | Mean | ♂♂ | ♀♀ | Mean | ♂♂ | ♀♀ | Mean | ♂♂ | ♀♀ | Mean | ♂♂ | ♀♀ | Mean |
| Group | | | | | | | | | | | | | | | | | | |
| 15–15 | 47 | 69 | 58 | 40 | 57 | 48·5 | 41 | 65 | 53 | 54 | 68 | 61 | 44 | 67 | 55·5 | 46 | 68 | 57 |
| 15–25 | 61 | 141 | 101 | 112 | 153 | 132·5 | 103 | 110 | 106·5 | 123 | 166 | 144·5 | 112 | 155 | 133·5 | 129 | 157 | 143 |
| 25–15 | 70 | 69 | 69·5 | 88 | 79 | 83·5 | 112 | 103 | 107·5 | 147 | 148 | 147·5 | 135 | 143 | 139 | 124 | 179 | 151·5 |
| 25–25 | 117 | 178 | 147·5 | 145 | 158 | 151·5 | 155 | 143 | 149 | 189 | 177 | 183 | 165 | 204 | 184·5 | 231 | 230 | 230·5 |

Each entry in the table represents the arithmetic mean for six individuals, with the following exceptions:

(i) Five **NFS** 15–25 females only; one female was crushed during transfer to the apparatus.

(ii) Five **K** 25–15 females only; one individual died in 5 min., and has been omitted from all calculations.

(iii) Three **B** 25–15 males, two **B** 25–25 males, and five **B** 25–25 females only. Great difficulty was experienced in obtaining sufficient **B** flies raised at 25° C. In all, twenty mass cultures were set up, in ten of which pupae were obtained. Many pupal deaths occurred, and the total number of flies emerging was:

| | Males | Females |
|---|---|---|
| Total emerged | 13 | 15 |
| Died before testing | 8 | 4 |
| Survived to test | 5 | 11 |

### A. *Physiological and developmental acclimatization*

In all three hybrid populations, flies which had been kept at 25° C., either as larvae or as adults survived at 33·5° C. for approximately 2·5 times as long as did the 15–15 group. Flies kept at 25° C. throughout lived longer than did flies which had been kept at 25° C. for only one period of their lives, but their survival time was less than would be expected if the increments due to acclimatization as a larva and as an adult were additive.

The acclimatization which results from keeping individuals at 25° C. during the larval stages is long-lasting, since the survival time is measured after the adults have been kept at 15° C. for approximately 7 days. In contrast, the acclimatization occurring during adult life is of short duration. **K/B** hybrids raised as larvae at 15° C., kept for 4 days after emergence at 25° C., and then for 2 further days before testing at 15° C., survived for

72 min. (mean for six males) and 82 min. (mean for six females), or only slightly longer than flies kept at 15° C. throughout.

There is therefore a good reason for regarding the processes of acclimatization occurring in larvae and in adults as different in kind. The long-lasting changes occurring during the larval stages will be referred to as 'developmental acclimatization', and the more transitory changes in adults as 'physiological acclimatization'. The nature of the changes involved is unknown, except that they probably reduce the rate of water loss, since there is evidence which suggests that the cause of death at 33·5° C. in dry air is the cumulative loss of water.

It has been assumed that the differences between the survival times of different groups of flies of the same genotype are due to a process of acclimatization occurring in individuals and not to selection acting through differential mortality. This assumption is certainly true as far as physiological acclimatization is concerned, since there were no deaths between emergence and testing in the hybrid populations, and very few in the inbred populations, except for **B** flies raised at 25° C. Mortality in the larval and pupal stages was not recorded, but it was probably small in the hybrid populations, although it may have been high in the inbred lines, and was certainly very high in the **B** line at 25° C. Therefore the evidence for developmental acclimatization in the **B** inbred line, and possibly in the other inbred lines, is based on a highly selected sample. However, it does not follow that the **B** flies which managed to emerge at 25° C. were selected on the basis of a genotype different from that of **B** flies emerging at 15° C. After thirty-five generations of brother-sister mating of a structurally homozygous stock, there is probably little genetic variability upon which selection could act. It seems more likely that the few survivors owed their success more to chance factors during development (i.e. factors into whose cause it is not at present efficient to inquire) than to their genotype.

However, if a differential mortality of flies of different genotypes at different temperatures is responsible for part of the difference between the 25–15 and 15–15 groups, its effect would be less in the $F_1$ hybrids than in the inbred lines, with their higher mortality. Yet, as will be shown, the extent of this difference is consistently greater in the hybrid than in the inbred populations, and there is in fact no significant difference between these groups in the **B** line, in which, on a selectionist interpretation, the greatest difference would be expected.

Summarizing, it is certain that the difference between the 15–25 and 15–15 groups is due to physiological acclimatization in individuals, and not to selection, and it seems safe to assume that at least the major part of the difference between the 25–15 and 15–15 groups is likewise due to developmental acclimatization in individuals.

### B. *Sex differences*

For all genotypes raised at 15° C., the females survived longer than did the males. However, the extent of developmental acclimatization was often greater in males than in females, so that, for flies which were kept at 25° C. as larvae, the sex difference is often reduced or absent.

### C. *Differences between inbred and hybrid populations*

There are no important differences between genotypes when kept throughout at 15° C. However, the question to be answered is whether there are differences in the extent of acclimatization, and this requires the choice of some measure of the extent. In the case,

for example, of physiological acclimatization, this could be taken either as the arithmetic difference between the mean survival times of the 15–25 and 15–15 groups, or as the ratio of the survival times of the two groups. The latter measure has been chosen; with the figures actually obtained, it provides a rather more stringent test of the significance of the differences observed.

The extent of physiological acclimatization, $R_p$, is therefore defined as the ratio of the survival times of the 15–25 and 15–15 groups, and of developmental acclimatization, $R_d$, as the ratio of the survival times of the 25–15 and 15–15 groups. These ratios, and their standard errors, were calculated as follows.

The logarithm of each individual observation was taken. If $x_i$, $y_i$ are the logarithms of the survival times of an individual male and female respectively, in the $i$th group, and for a given genotype, then the mean logarithmic survival time of flies in the $i$th group is

$$\tfrac{1}{2}(\bar{x}_i+\bar{y}_i)=\tfrac{1}{12}\{\Sigma x_i+\Sigma y_i\}. \quad (1)$$

A measure of the within-group variance for this genotype is given by

$$v=\tfrac{1}{40}\Sigma_i\{\Sigma(x_i-\bar{x}_i)^2+\Sigma(y_i-\bar{y}_i)^2\}. \quad (2)$$

For the three hybrid populations, this estimate is based on 40 d.f., 5 for males and 5 for females in each of 4 groups. A smaller number of degrees of freedom is available in the inbred populations, because not all samples contained six flies.

If the extent of acclimatization is measured by the ratio $R$ of the survival times in the $i$th and $j$th groups, then

$$\log R=\tfrac{1}{2}(\bar{x}_i+\bar{y}_i)-\tfrac{1}{2}(\bar{x}_j+\bar{y}_j), \quad (3)$$

from which the values of $R$ in Table 2 were calculated. The error variance of the estimate is $\frac{1}{6}v$, whence it is possible to calculate the standard errors of log $R$ and of $R$.

Table 2. *Extent of acclimatization, and variability in a single environment*

| Genotype | Extent of physiological acclimatization $R_p$ | Extent of developmental acclimatization $R_d$ | Variability in a single environment: Sum of squares | Degrees of freedom | Variance |
|---|---|---|---|---|---|
| **B** | 1·66±0·21 | 1·19±0·16 | 0·5515 | 32 | 0·01724 |
| **NFS** | 2·82±0·25 | 1·81±0·16 | 0·3251 | 39 | 0·00834 |
| **K** | 2·09±0·15 | 2·06±0·15 | 0·2253 | 39 | 0·00578 |
| **NFS/B** | 2·36±0·14 | 2·38±0·14 | 0·1630 | 40 | 0·00408 |
| **K/B** | 2·42±0·16 | 2·55±0·17 | 0·2010 | 40 | 0·00503 |
| **NFS/K** | 2·59±0·15 | 2·69±0·16 | 0·1366 | 40 | 0·00342 |

Table 2 gives the measures of physiological and of developmental acclimatization for the six genotypes. A value of $R=1{\cdot}0$ would imply that no acclimatization had occurred. The value $R_d=1{\cdot}19$ for developmental acclimatization in the **B** line is not significantly different from unity. Apart from this, the lowest value is $R_p=1{\cdot}66$ for physiological acclimatization in the **B** line. In this case, log $R=0{\cdot}221$, $\frac{1}{6}v=0{\cdot}00287$, and hence the Student–Fisher $t=0{\cdot}221/0{\cdot}0535=4{\cdot}12$, so that the significance of acclimatization is in no doubt.

In the case of physiological acclimatization, there are no consistent differences between inbred and outbred flies. The greatest extent of acclimatization was shown by the **NFS** line; this is mainly due to the short survival time of the 15–15 group rather than to a long survival of the 15–25 group. If the extent of acclimatization is measured by the

arithmetic difference in survival times, the **NFS** line closely resembles the **NFS/K** and **NFS/B** hybrids. The least acclimatization was also found in an inbred line, the **B** line. As Table 1 shows, acclimatization was normal in **B** females, but slight in **B** males.

In the case of developmental acclimatization, however, there are consistent differences between inbred and outbred populations. The extent of acclimatization is lower in the three inbred lines than in any of the hybrids. In the **B** line, the extent is small, and in fact is not statistically significant. In the **NFS** line it is significantly lower than in any of the hybrids. In the **K** line the extent of developmental acclimatization, although not significantly different from that in **NFS/B** hybrids, is significantly less ($P=0{\cdot}05$) than that in either of the hybrids having **K** flies as one parent.

This difference in the capacity for developmental acclimatization between inbred and outbred flies is illustrated in Fig. 1, which shows the survival times of the 15–15 and 25–15 groups of the **B** and **NFS** inbred lines, and of hybrids between them.

### D. *Variability within groups*

Equation (2) gives an estimate of the variability of flies of a given sex and genotype, which have been raised as nearly as possible in the same conditions. The estimate is, at least approximately, independent of the mean survival time. Estimates of the within-group variance are given in Table 2.

There is an almost exact correspondence between the presence in a population of a high capacity for developmental acclimatization and a low within-group variability. With such small samples, only rather striking differences in variability are significant; with 40 d.f. in each class, a variance ratio $F=1{\cdot}69$ is significant at the 0·05 probability level. Thus most of the differences in variability in Table 2 are not significant. However, the **B** line, which can scarcely acclimatize as a larva, is significantly more variable than any other, and the **NFS** line significantly more variable than either of the hybrids **NFS/B** or **NFS/K**. Thus, although the **K** line, the most adaptable of the inbred lines, does not differ significantly in variability from the hybrids, there is no doubt that the general correspondence between a high capacity for developmental acclimatization and a low variability is statistically significant.

## 4. Discussion

The greater fitness often found in outbred organisms has been explained by a number of authors in terms of their greater capacity for regulation during development. This capacity has been inferred from the constancy of adult phenotype, although it has been recognized that regulation must imply change as well as constancy. For example, a thermostat implies some method of changing the output of the heating or cooling units; homoiothermy in man requires changes in the rates of shivering and sweating. A more concrete picture of developmental homoeostasis will be possible when we have studied not only those features of an organism which are regulated and kept constant, but also those whose change has a regulative function; by analogy, when we have measured the rate of sweating as well as the body temperature. The present work is an attempt in that direction.

The time for which a fly can survive at a high temperature is an aspect of its phenotype, but one which changes according to the individual's previous environmental history.

These changes can be regarded as regulative, in the sense that individuals which have been reared at 25° C. are better able to preserve their life and function when exposed to a high temperature than are those reared at 15° C. It is therefore a satisfying confirmation of the hypothesis outlined in the introduction to this paper to find that those genotypes

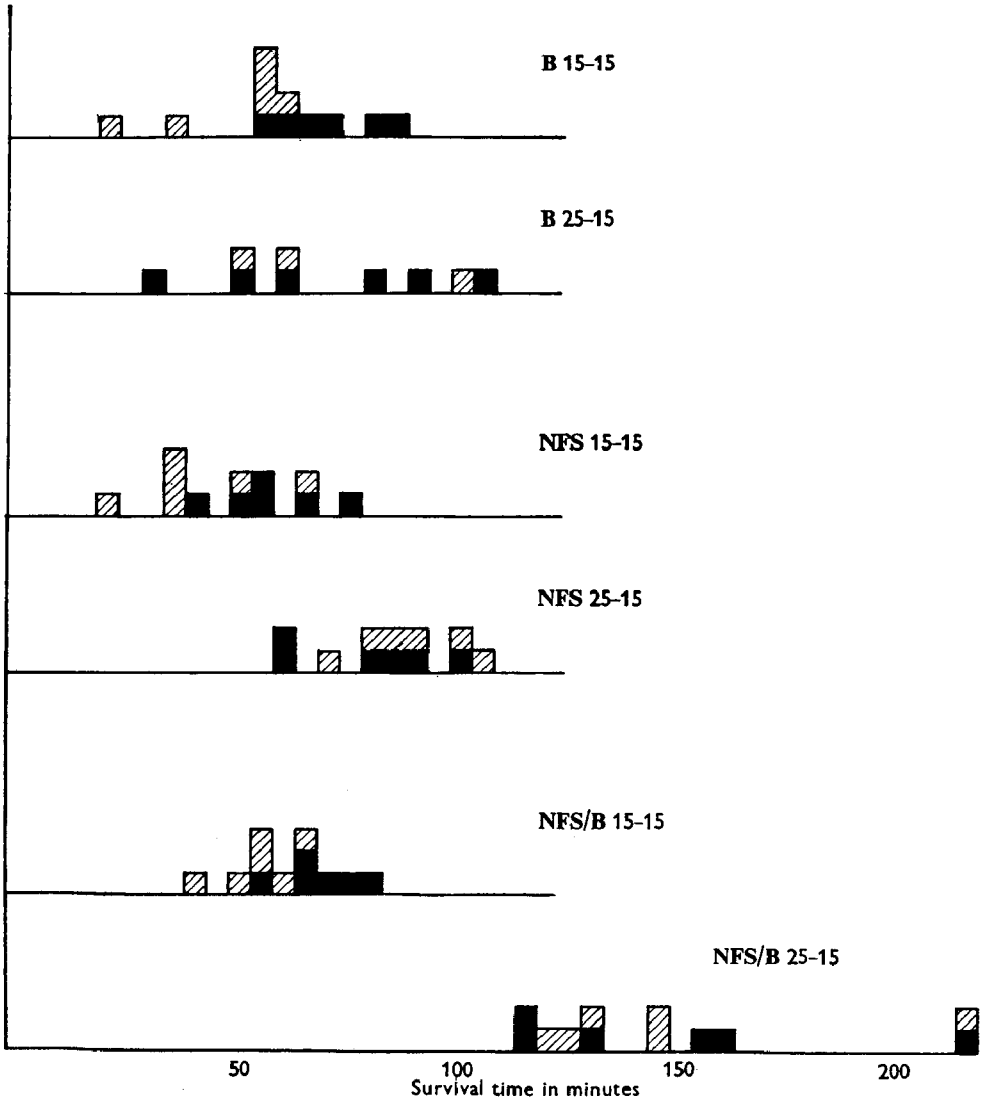


Fig. 1. Survival time in minutes at 33·5° C. in dry air of the B and NFS inbred lines, and of hybrids between them. Each square represents one individual; hatched, male; black, female. 15–15, kept at 15° C. throughout; 25–15, kept at 25° C. until emergence, and subsequently at 15° C.

which showed the greatest degree of constancy when raised at a given temperature also showed the greatest extent of developmental acclimatization when raised at different temperatures.

It is interesting to compare this result with those obtained in a study of developmental homoeostasis in *D. pseudoobscura* by Dobzhansky & Levene (1955). They measured the

viability, i.e. the percentage survival to emergence, in a number of homozygous and heterozygous genotypes in several environments. Those genotypes with the highest average viability, usually heterozygotes, also showed the least change in viability in different environments. Further, those genotypes which showed a high and uniform viability in different environments also showed the least variation in viability in a given environment, as measured by differences between replicate cultures. Formally, this latter observation is the opposite to that obtained in my work, in which a small variation in a given environment was associated with large differences between individuals raised in different environments. There is, however, no contradiction. Dobzhansky & Levene were studying a character for which fitness required constancy; the 'target' is always 100% viability. For the character studied in this paper, fitness requires a change in a changed environment.

Differences in developmental flexibility, the term used by Thoday (1953) for a capacity for developmental homoeostasis, are most easily studied, at least in *D. subobscura*, by comparing inbred and outbred populations, since inbreeding usually leads to a striking decline in vigour. However, it is clear from the present work that differences in developmental flexibility are not solely due to differences in the degree of genetic heterozygosity, since there were appreciable differences between the inbred lines, one of which, the **K** line, approached the hybrid populations in its capacity for regulation. Hence the capacity to regulate during development does not necessarily depend on the presence of two alleles at a given locus with different levels of activity in different environments, although the general superiority of outbred flies suggests that such a mechanism may play a part in regulation.

## 5. Summary

Three inbred lines of *D. subobscura*, and the three types of $F_1$ hybrid between them, were reared at two temperatures, 15 and 25° C., and the time for which adult flies could survive at 33·5° C. in dry air recorded.

Individuals which had been kept at 25° C. either as larvae or as adults survived for much longer than those kept at 15° C. throughout. Two kinds of temperature acclimatization can occur: a long-lasting 'developmental acclimatization' in individuals kept at 25° C. until emergence, and subsequently at 15° C., and a short-lived 'physiological acclimatization' in individuals kept at 25° C. as adults only.

There were no consistent differences between the capacities for physiological acclimatization of inbred and outbred flies, but outbred flies showed a significantly greater extent of developmental acclimatization than did inbred ones. Those genotypes which showed the greatest extent of developmental acclimatization were also the least variable when raised at a given temperature.

These results confirm the hypothesis that organisms which show a greater capacity for regulation during development, as expressed by greater constancy of adult phenotype, are also capable of greater adaptive modification of phenotype in response to changed conditions.

## REFERENCES

Dobzhansky, Th. & Levene, H. (1955). Genetics of natural populations. XXIV. Developmental homoeostasis in natural populations of *Drosophila pseudoobscura*. *Genetics*, **40**, 797.

Haldane, J. B. S. (1948). Symposium sui fattori ecologici e genetici della speciazioni negli animali. *Ric. sci. Supp. A.*, p. 54.

LERNER, I. M. (1954). *Genetic homoeostasis.* Edinburgh: Oliver and Boyd.

MAYNARD SMITH, J., CLARKE, J. M. & HOLLINGSWORTH, M. J. (1955). The expression of hybrid vigour in *Drosophila subobscura*. *Proc. Roy. Soc.* B, **144**, 159.

MELLANBY, K. (1939). Low temperatures and insect activity. *Proc. Roy. Soc.* B, **127**, 473.

MICHIE, D. & MCLAREN, A. (1955). The importance of being cross-bred. *New Biol.* **19**, 48.

ROBERTSON, F. W. & REEVE, E. C. R. (1952). Heterozygosity, environmental variation and heterosis. *Nature, Lond.*, **170**, 296.

THODAY, J. M. (1953). Components of fitness. *Symp. Soc. Exp. Biol.* **7**, 96.

WADDINGTON, C. H. (1942). Canalization of development and the inheritance of acquired characters. *Nature, Lond.*, **150**, 563.