Genet. Res., Camb. (1965), **6**, *pp.* 1–12
With 2 text-figures
Printed in Great Britain

The effect of inbreeding on temperature acclimatization in *Drosophila subobscura*

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(*Received* 15 *October* 1963)

1. INTRODUCTION

The results of an investigation into the effect of inbreeding on the rates of gain and loss of acclimatization to temperature and on the degree of acclimatization attained are reported here. Previous work on other physiological characteristics such as rate of development, fertility and longevity in inbred and outbred flies of this species, reviewed by Maynard Smith, Clark & Hollingsworth (1955), lead us to expect that hybrid flies would exhibit both a greater degree and a higher rate of temperature acclimatization than inbred flies. This is because hybrids exhibit hybrid vigour, such vigour leading to an increase in fitness. Fitness can be measured by the ability to survive in a wide range of environments, for example, high viability (Dobzhansky & Levene, 1955). At other times the ability to change the phenotype in a changing environment may confer fitness, as, for example, the ability to acclimatize to different environmental temperatures. Both these facilities have previously been studied with respect to changes in the phenotype occurring during development and have been ascribed to 'developmental flexibility' (Thoday, 1953) or 'developmental homeostasis' (Lerner, 1954).

Acclimatization to temperature in *Drosophila subobscura* has been studied by Maynard Smith (1956, 1957). He was able to show that hybrid flies were capable of developmental acclimatization to a greater extent than inbred flies. Our hypothesis was that the greater fitness of hybrids should be demonstrable by a greater capacity to regulate after development has been completed, that is as adults, in addition to being able to regulate during development. The adaptive value of the capacity of physiological acclimatization to temperature by adult *Drosophila* in a natural environment with a fluctuating diurnal and seasonal temperature need not be emphasized.

We did not further consider developmental acclimatization to temperature at this time because it is undoubtedly a more complex process than physiological acclim-

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atization. More importantly, we could not be certain of having excluded selection within our cultures. That is, flies in any one line, which emerge after developing at one temperature could be survivors and be genetically different from the survivors raised at another temperature. Maynard Smith (1956) reported high pre-adult mortality in the inbred lines raised at a high temperature.

In this study we have used changes in the mean heat-death points as our measure of temperature acclimatization. We have followed Precht (1957) in distinguishing between acclimatization to temperature as determined within viable limits (Leitsungsadaptation), and acclimatization as determined by the ability to withstand exposure to lethal temperatures (Resistenzadaptation). We have chosen this latter form of temperature acclimatization for our present study.

By temperature acclimatization we mean the physiological alterations which occur in individuals which result in changes in their heat-death points. The heat-death point is the time taken for an individual to die at a lethal temperature, that is, its survival time at the lethal temperature.

Our expectations have only been partially fulfilled. For instance, we found that the hybrid flies were unable to adapt to any greater extent than the inbred flies. But we did find that all hybrid flies lost their acclimatization to temperature more quickly than inbred flies, and that, with the exception of the K/B males, the hybrids gained acclimatization to temperature more quickly than inbred flies.

2. MATERIALS AND METHODS

The flies used were males from two inbred lines, B and K, and males from the reciprocal hybrids between these inbred lines K♀ × B♂ (K/B) and B♀ × K♂ (B/K). Female flies were not used because their physiology as adults is known to be different from that of males. This is largely because of the stress of egglaying (Maynard Smith, 1958). The inbred lines are structurally homozygous for all chromosomes, but for different orders. They have been maintained on a standard maize-meal medium by brother-sister matings for over 100 generations. During this time they have been kept at around 20°C. and during the course of these experiments, some twelve generations, at 18·5°C. ± 0·3°C. in a temperature-controlled cabinet.

Flies were removed from the culture bottles daily and left in standard yeasted food vials for at least 1 day at 18·5°C. to allow completion of cuticular hardening. They were then transferred, in vials, to one of two acclimatization cabinets, which were kept at 24·8°C. ± 0·5°C. (referred to as 25°C. below and in the tables and figures) and 15°C. ± 0·3°C. respectively with bimetal thermostats and 'Sunvic' controls. The 15°C. cabinet was kept in a cold-room. The humidity in the cabinets was not controlled. The flies were transferred to fresh food vials twice weekly. Regular transfer is important, particularly at the higher acclimatization temperature, for the food medium soon dried and shrinks. It also deteriorates and is then less able to support the flies. Recent observations have suggested that this is largely due to depletion of the yeast supply.

In the experiments to determine the rates of gain and loss of acclimatization

flies were kept at the first acclimatization temperature until fully acclimatized at that temperature. Trials had shown that this was approximately 5 days at 15°C. and 2 days at 25°C. They were transferred to the second acclimatization temperature for varying lengths of time, from 6 hours to 10 days, before being exposed to the lethal temperature, 34°C., in dry air.

In the experiments to determine the extent of acclimatization attained, all flies were fully acclimatized at the two acclimatization temperatures, as described above, and then transferred to one of four lethal temperatures, 33°C., 34°C., 35°C., 36°C. in dry air.

The heat-death point (survival time) at these lethal temperatures was measured by immersing the flies in a water bath kept to within $\pm$ 0·1°C. with a contact thermometer and a 'Sunvic' control. The flies were placed individually in one of the twenty-six 3 in. × 1 in. vials attached to a rack. Air, dried over calcium chloride, was pumped through the vials in series, the dryness of the air in each vial being indicated by a piece of cobalt chloride paper. Trials showed that the air in the vials containing the flies took 12 min. to reach water-bath temperature and consequently observations were begun 12 min. after immersion of the rack.

It was not possible to design an experiment in which all the flies were reared and treated together. It was found that no more than twenty-six flies could be conveniently examined in the 5-min. periods between observations. In consequence of this some of the mean values presented in Tables 1 and 2 are obtained by combining observations made on different occasions over a period of about a year. Although the conditions under which the flies were bred, kept and tested were standardized and not known to vary throughout the duration of the investigation, some of the observed variations in survival times are undoubtedly the result of causes beyond our control. This is more likely to be true with respect to the inbreds which are known to be more susceptible to environmental variations than hybrids. Nevertheless, we feel justified in believing that the mean survival times given in Tables 1 and 2 are meaningful. Table 3 gives a typical breakdown of the mean figures in Table 1 for the 3-day acclimatized flies. It shows that, particularly when the number of observations exceeds twenty, the differences between the component means is small enough for us to assume that the sample mean values given in Tables 1 and 2 are representative.

Two methods are generally used in determining survival times at lethal temperatures for a given species. Animals may either be taken abruptly from the acclimatization temperature to the lethal temperature or they may be raised from the acclimatization temperature to the lethal temperature over a period of time. Both these methods have been criticized. In the first method the sudden rise of temperature may create a massive shock to the animals (Spoor, 1955). In the second method additional acclimatization may occur (Bovee, 1949). In the present study the initial shock experienced by the flies, when they fell to the bottom of the tube and lay still for several minutes, was not so intense as to mask the acclimatization effect, and the 12 min. heating-up period was insufficient to allow any measureable acclimatization to occur.

Any fly found dead in a 5-min. interval was taken as dying at the previous class mid-value. Our criterion of death was the cessation of all leg movement, even on shaking the vials, but the time of death was not recorded until confirmed by two further observations. At first the flies were very active but soon went into a shocked condition. After recovery from shock the flies either stood still or walked about slowly and, with increasing frequency, fell onto their backs as they approached the heat-death point. They were eventually unable to right themselves and death then usually followed quickly.

3. RESULTS

(i) *The rates of gain and loss of acclimatization to temperature*

Figure 1 *a* shows the rate at which 15°C. acclimatized K/B, B/K, B and K males gain acclimatization to 25°C. The rates of loss of acclimatization to 25°C. of these hybrid and inbred flies is shown in Fig. 1 *b*. The mean survival times of these flies are given in Table 1. B/K males gain acclimatization to temperature more quickly than any other group, it being completed in about 15 hours. In K males acclimatization takes 24 hours whilst it takes some 2 days in K/B and B males. It will be noticed that the mean survival times of B males falls off with time at 25°C., until after 10 days at 25°C. it is below that of 15°C. acclimatized flies. A similar but smaller fall will also be noticed in K males at 25°C. We interpret this as an ageing phenomenon which is at present undergoing further investigation. Figure 1 *b* also shows that B/K males lose acclimatization to 25°C. when at 15°C. in about 2 days, which is much quicker than in the other groups of flies, where it is 4 days for K/B males, 5 days for B males and 6 days for K males.

(ii) *The extent of acclimatization attained*

Table 2 gives the mean survival times of 15°C. and 25°C. fully acclimatized K/B, B/K, K and B males at four lethal temperatures. Of the hybrids the K/B males have the higher mean survival times at all lethal temperatures in both the 15°C. and 25°C. acclimatized groups. In the inbreds both the 15°C. and 25°C. acclimatized K males survive longer than do the B males, in fact the K males have survival times very similar to the B/K males. The similarity of the mean survival times of all flies at 36°C. indicates that this temperature is close to the upper lethal limit (Fry, Hart & Walker, 1946) of this species. The upper lethal limit can be defined as that temperature-time relationship which cannot be increased by raising the acclimatization temperature. As measured by their survival times, the K/B males were the fittest and the B males the least fit of those tested.

The coefficients of variation of the survival times, which range from 0·09 to 0·42, have an average value of 0·19. The value of 0·26 for all the heat-death times at 36°C. shows that these survival times are relatively the most variable in spite of the larger variances at the lower lethal temperatures. This is because at 36°C. the method of determining the heat-death point is insufficiently delicate, the 5-min. observation

periods being long relative to the survival time. To have recorded individual heat-death points at this temperature would have changed our criteria and so possibly made the comparisons unfair.

Figure 2 shows the survival times given in Table 2 plotted as logarithms. The

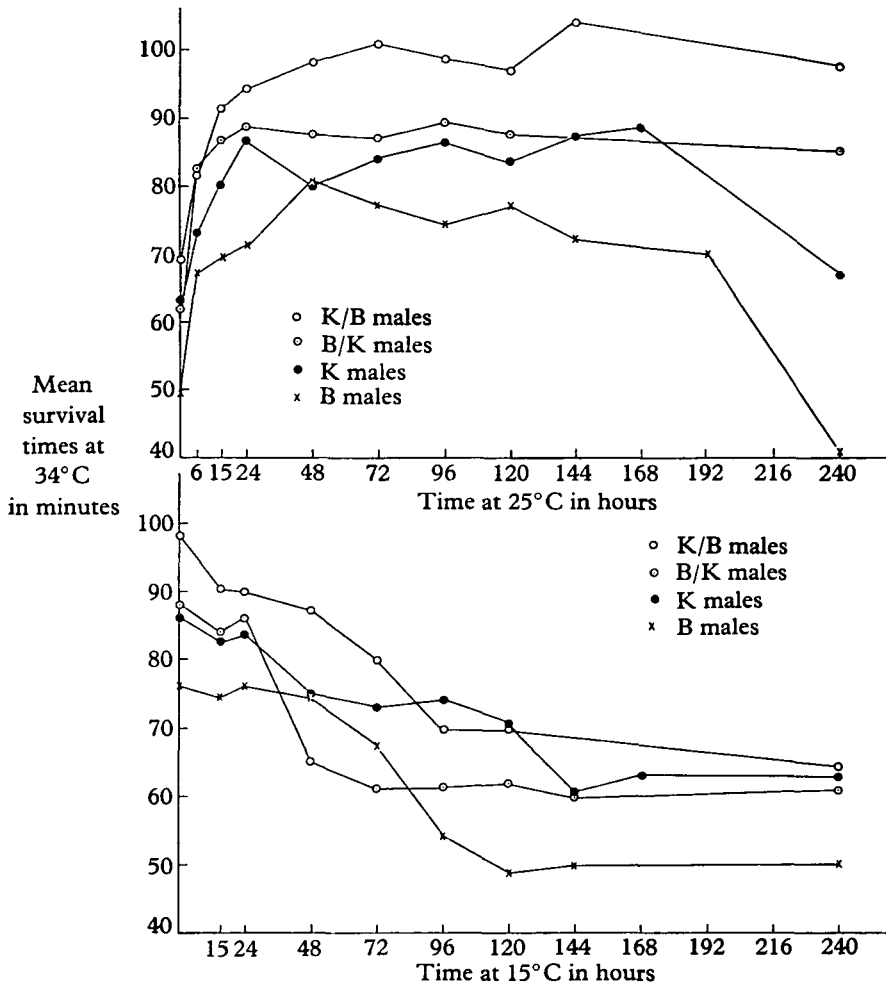

Fig. 1. The rates of gain and loss of acclimatization to temperature by inbred and hybrid *D. subobscura* males as measured by their survival times at 34°C. after spending various times at (*a*) 25°C., (*b*) 15°C.

extent of acclimatization attained is shown by the distance the two curves are apart, no acclimatization occurring if the two curves coincide. The figure suggests that there is little difference in the extent of acclimatization in any of the four groups of flies. An analysis of covariance of survival times on lethal temperature of these flies is shown in Table 4. The analysis has been calculated from the logarithms of the survival times of the individual flies. It shows no significant departures from

linearity in any of the eight lines. For the K/B, B/K and K flies there is no significant departure from parallelism and the table shows the combined regression coefficients. For the B flies the slope at 25°C. is significantly steeper than at 15°C., as is clearly seen in Fig. 2, and separate regression coefficients are given. The convergence of

Table 1. *The mean survival times (S.T.) at 34°C. of 15°C. and 25°C. fully acclimatized inbred and hybrid males after reacclimatization at the second temperature for the stated periods of time*

A. Gain of acclimatization to temperature (15°C → 25°C).

Time spent at 2nd acclimatization temperature (25°C.)	K/B		B/K		K		B	
	S.T.	n	S.T.	n	S.T.	n	S.T.	n
6 hours	80·0 ± 1·6	26	82·2 ± 1·1	51	73·0 ± 2·0	24	67·2 ± 2·4	37
15 hours	91·6 ± 1·5	59	86·8 ± 2·0	29	82·3 ± 2·1	33	69·8 ± 3·1	19
1 day	94·3 ± 1·0	44	88·7 ± 1·6	21	87·0 ± 2·5	41	70·9 ± 2·9	22
2 days	98·1 ± 1·4	37	87·7 ± 1·5	39	80·3 ± 1·9	20	80·5 ± 1·8	87
3 days	100·7 ± 1·2	35	87·5 ± 1·5	47	84·1 ± 1·5	32	76·8 ± 1·9	38
4 days	98·3 ± 2·2	32	89·4 ± 1·5	29	86·3 ± 1·4	39	74·6 ± 3·3	37
5 days	96·9 ± 1·5	23	87·6 ± 1·4	13	83·9 ± 1·6	22	76·6 ± 2·6	22
6 days	104·3 ± 2·1	22	—	—	87·8 ± 2·0	26	71·7 ± 2·1	35
7 days	—	—	—	—	88·5 ± 1·8	9	—	—
8 days	—	—	—	—	—	—	70·1 ± 2·6	19
10 days	97·3 ± 1·6	14	84·6 ± 1·6	44	67·3 ± 4·6	22	41·2 ± 3·3	14

B. Loss of acclimatization to temperature (25°C → 15°C).

Time spent at 2nd acclimatization temperature (15°C)	K/B		B/K		K		B	
	S.T.	n	S.T.	n	S.T.	n	S.T.	n
15 hours	90·3 + 3·1	22	83·3 ± 2·6	18	82·8 ± 1·9	35	74·3 ± 2·5	32
1 day	89·9 ± 3·1	18	86·2 ± 2·2	17	83·2 ± 1·8	25	75·8 ± 4·6	14
2 days	87·5 ± 1·6	33	65·0 ± 1·3	30	75·2 ± 1·7	51	73·8 ± 3·4	19
3 days	79·9 ± 1·7	36	61·1 ± 1·0	19	72·8 ± 1·7	51	67·0 ± 3·6	23
4 days	68·9 ± 1·3	32	61·6 ± 2·0	18	73·9 ± 2·1	39	54·1 ± 1·8	26
5 days	69·1 ± 2·2	27	62·1 ± 2·4	18	70·6 ± 1·8	38	48·7 ± 2·1	23
6 days	—	—	59·8 ± 2·4	16	60·5 ± 2·2	20	49·8 ± 2·5	19
7 days	—	—	—	—	63·6 ± 1·6	42	—	—
10 days	63·8 ± 1·6	18	60·7 ± 3·5	11	62·7 ± 2·5	24	50·1 ± 3·1	21

these lines is taken as indicating that the temperatures used are close to the upper lethal limit of the B flies. One would expect similar convergence for all flies at lower temperatures for here, where the survival time is of the order of days, further reacclimatization would occur in both 15°C. and 25°C. acclimatized flies before death occurred.

Table 2. *Mean survival times (S.T.) in minutes, with standard errors, at four lethal temperatures of 15°C. and 25°C. acclimatized hybrid and inbred males*

Lethal temp. (°C.)	Acclim. temp. (°C.)	K/B S.T.	K/B n	B/K S.T.	B/K n	K S.T.	K n	B S.T.	B n
33	25	223·2 ± 3·9	24	162·1 ± 2·9	49	160·9 ± 4·8	20	148·9 ± 5·3	21
	15	118·5 ± 3·4	18	97·1 ± 3·0	66	105·4 ± 3·5	52	67·6 ± 2·7	40
34	25	98·2 ± 0·7	207	87·2 ± 0·7	222	84·7 ± 0·8	222	75·5 ± 0·9	276
	15	67·8 ± 1·1	77	62·5 ± 1·0	82	63·3 ± 1·2	86	49·5 ± 1·5	62
35	25	58·2 ± 1·2	22	52·4 ± 1·5	25	48·3 ± 1·1	18	47·2 ± 1·9	18
	15	29·8 ± 1·1	38	28·2 ± 1·2	22	33·3 ± 1·6	18	29·8 ± 1·9	22
36	25	31·6 ± 1·3	14	31·8 ± 1·2	21	23·5 ± 1·0	20	27·4 ± 2·7	18
	15	19·7 ± 1·8	12	18·2 ± 0·7	42	13·3 ± 0·7	49	16·7 ± 1·2	12

Table 3. *A typical breakdown of the combined means of Table* 1. *Survival times in minutes*

	Temperature °C.	Combined mean	n	Component mean 1	n	Component mean 2	n	Component mean 3	n	Component mean 4	n
B/K											
3 day	15→25	87·5 ± 1·5	47	93·7 ± 3·0	7	83·3 ± 1·6	16	88·4 ± 2·3	24	—	—
K/B											
3 day	15→25	100·7 ± 1·2	35	100·5 ± 2·0	12	100·8 ± 1·2	23	—	—	—	—
	25→15	79·9 ± 1·7	36	80·15 ± 1·9	20	78·0 ± 3·1	16	—	—	—	—
K											
3 day	15→25	84·1 ± 1·5	32	82·6 ± 2·6	13	85·1 ± 1·8	19	—	—	—	—
	25→15	72·8 ± 1·7	51	68·6 ± 5·7	8	67·1 ± 2·2	11	76·6 ± 2·7	11	75·1 ± 2·8	21
B											
3 day	15→25	76·8 ± 1·9	38	78·0 ± 2·5	22	75·2 ± 2·7	16	—	—	—	—
	25→15	67·0 ± 3·6	23	73·7 ± 5·0	14	64·1 ± 4·8	9	—	—	—	—

Table 4. *Analysis of covariance of the regression of the logarithms of survival times on temperature at four lethal temperatures (33°C., 34°C., 35°C. and 36°C.) for K/B, B/K, K and B flies acclimatized at 25°C. and 15°C.*

	Regression coefficients and standard errors	Acclimatization indexes and standard errors
K/B 25°C. and 15°C. averaged	0·286 ± 0·005	0·199 ± 0·007
B/K 25°C. and 15°C. averaged	0·243 ± 0·004	0·201 ± 0·007
K 25°C. and 15°C. averaged	0·301 ± 0·005	0·183 ± 0·009
B 25°C.	0·243 ± 0·010	(0·219 ± 0·011)*
15°C.	0·193 ± 0·011	

* See text.

https://doi.org/10.1017/S0016672300003931 Published online by Cambridge University Press

The acclimatization index is defined as the difference between adjusted means, that is, the vertical distance between parallel lines. It is shown in Table 4 for the K/B, B/K and K flies. In the B flies, where the lines are not parallel, the acclimatiz-

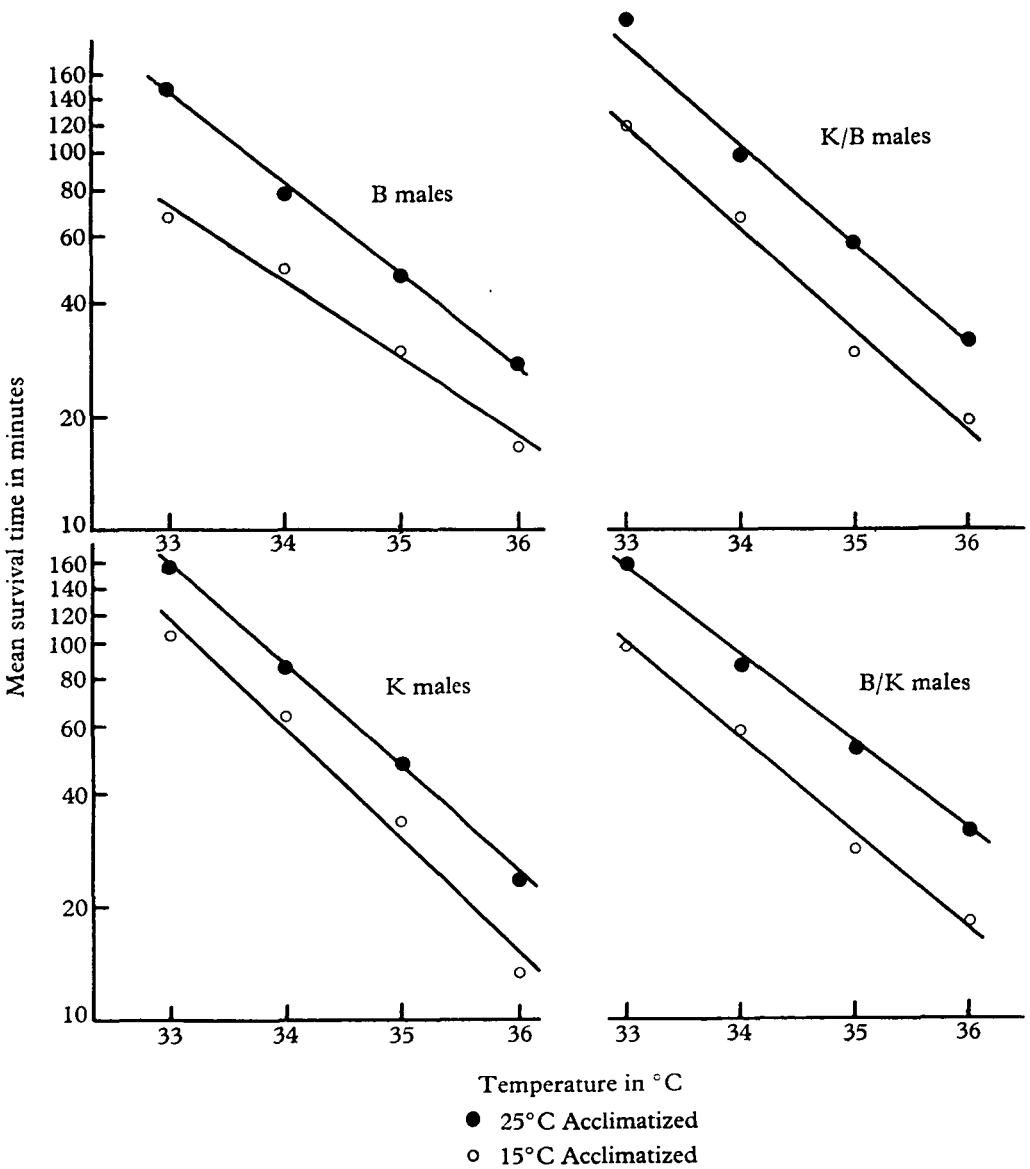

Fig. 2. The extent of acclimatization to temperature by inbred and hybrid *D. subobscura* males as measured by the differences in their survival times at four lethal temperatures.

ation index cannot be defined in this way as it would depend on the arbitrary choice of the numbers of flies tested, but, for the sake of comparison, it is given in the above table. If the three slopes in the K/B, B/K and K flies are compared, an analysis of

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variance shows that there are significant differences between them ($p < 0{\cdot}001$). On the other hand there are no significant differences between the three acclimatization indexes ($p < 0{\cdot}05$).

4. CONCLUSIONS

The results in Fig. 2 and Table 4 show that the ability to acclimatize to temperature is not less in the inbred flies than in the hybrids. Although the inbred lines have been brother-sister mated for more than 100 generations and both are known to be structurally homozygous, it does not mean that they are homozygous for all loci, for a balanced polymorphism may be involved. The viability of the inbred lines is low, particularly that of the B line. That those zygotes which survive to be adults have the ability to acclimatize to temperature suggests that this function is a prerequisite of survival and is as fundamental a function to the fly as its ability to digest its food. In this respect the effect of inbreeding on temperature acclimatizaton

Table 5. *Ratios of mean survival times of* 15°C. *and* 25°C. *acclimatized flies.* (*A ratio of* 1·00 *would indicate that acclimatization had not occurred*)

	33°C.	34°C.	35°C.	36°C.
K/B	1·88	1·45	1·95	1·60
B/K	1·67	1·40	1·86	1·75
K	1·53	1·38	1·45	1·77
B	2·20	1·53	1·58	1·64

differs from that on other physiological characters studied in this species, such as rate of development, fertility, and longevity (Maynard Smith, Clarke & Hollingsworth, 1955).

Maynard Smith (1956, 1957) has shown that hybrid *D. subobscura* exhibited a greater degree of temperature acclimatization than did inbred flies, we were unable to confirm this observation. Our work, however, is not strictly comparable with that of Maynard Smith for the following reasons. First, he combined his study of physiological acclimatization with developmental acclimatization. It was only in the latter type of acclimatization that he was able to demonstrate any consistent differences between hybrids and inbreds. Secondly, he did not control his acclimatization temperatures accurately. In one case the temperature fluctuated over a range of 4°C. In our opinion flies acclimatized at the extremes of this range would have different mean survival times for, as we have shown, acclimatization to temperature can be attained very quickly.

Our work also differs in the method of expression of the extent of acclimatization attained. Maynard Smith expressed his results as a ratio of the mean survival times of his acclimatized group. Our results show that this is an unsatisfactory method, since such ratios vary over a range of lethal temperatures (see Table 5).

Maynard Smith restricted his investigation to the amount of acclimatization gained or lost and did not consider the rates of gain or loss. Figures 1*a* and 1*b* show that in this latter respect hybrids are fitter than inbred flies, B/K males being in this

respect the most fit of all. The difference in rate at which temperature acclimatization is gained and lost in poikilothermic animals is a long-known phenomenon, and can best be explained by the fact that temperature acclimatization involves metabolic processes which take place more quickly at high than at low temperatures.

The higher survival times of K males and the superior survival of the K/B males indicate the considerable importance of the K X-chromosomes. It is not surprising that such an effect could be detected, for the X-chromosome constitutes approximately one-fifth of the genotype in this species.

The cause of death of poikilothermic animals at high temperatures is not understood, though a variety of causes have been suggested (see Heilbrunn, 1952, and Giese, 1962, for reviews). It should perhaps be pointed out that the factors that bring about heat-death will not necessarily be the same in different species, and also

Table 6. *Temperature coefficients* (Q_{10}) *and Arrhenius* μ *of heat death in dry air of* 15°*C. and* 25°*C. acclimatized inbred and hybrid* D. subobscura *males*

	Temperature °C.	Q_{10} (33°C.–36°C.)	Arrhenius μ cal./mole
K/B	15	398	113,100
	25	676	123,100
B/K	15	265	105,400
	25	229	102,600
K	15	1000	130,500
	25	687	121,000
B	15	105	88,000
	25	286	108,300

that these factors are subject to alteration, as shown by the mobility of the heat-death point. It is also evident that the causes of heat death must be closely related to the factors involved in temperature acclimatization. This point is further borne out when the temperature coefficients Q_{10} and Arrhenius μ of heat death, protein and enzyme denaturation are compared. Values of Q_{10} and Arrhenius μ for the inactivation of enzymes are characteristically high. Neilands & Stumpf (1955) quote a range of μ from 40,000 cal./mole to 100,000 cal./mole. Giese (1962) gives Q_{10} for egg albumen coagulation of 635 (μ = 150,000 cal./mole) and for haemoglobin coagulation a value of 13·8 (μ = 60,000 cal./mole).

Although the flies were subjected to desiccation as well as high temperature in our experiments, the very high values of Q_{10} and μ we obtained (see Table 6) make it very unlikely that desiccation is the primary cause of death, though it may be an important secondary factor as was suggested by Maynard Smith (1957). We suggest that enzyme denaturation is the fundamental reason for the death of the flies at the lethal temperatures used.

As stated above the factors involved in heat death and also in temperature acclimatization are closely related. Several workers in the field of temperature physiology have shown that temperature acclimatization involves changes in enzymes such as

catalase, succinate dehydrogenase (Precht, Christophersen & Hensel, 1955; Suhrmann, 1955), cytochrome-C (Stangenberg, 1955). More recently Kanugo & Prosser (1959) have postulated that temperature acclimatization in the goldfish involves quantitative changes in several enzyme systems.

In *Drosophila* enzyme changes during acclimatization could be either quantitative or qualitative, or both. Haldane (1948) and Robertson & Reeve (1952) have suggested that hybrids would exhibit 'biochemical versatility' because, being heterozygous, they have a greater diversity of alleles and hence more potential ways of producing enzymes. Our results suggest that if a new environmental temperature calls for a new enzyme (or enzymes) or changes in existing enzymes, both inbred and hybrid *D. subobscura* can, if given time, produce them. But hybrids, having a greater 'versatility', can conform to the new requirements more quickly. Thus the delay in the inbred flies may be due to a temporary imbalance between demand and production.

Our results (Fig. 1 *a*) also show that aged inbred flies are more susceptible to high lethal temperatures than young flies. Preliminary studies, not presented here, also indicate that aged inbred and hybrid males acclimatize only to a small extent. These results suggest that during ageing changes have taken place and that these changes are affecting enzyme production and activity. Maynard Smith (1962) has also suggested that ageing in *Drosophila* may be associated with an inability to maintain a steady state of protein metabolism. We are using these observed phenomena in further studies in the hope of shedding light upon both ageing and acclimatization.

SUMMARY

1. Rates of gain and loss of acclimatization to temperature of males from two inbred lines and the hybrids between them were measured by recording their survival times in dry air at a lethal temperature (34°C.).

2. All hybrid males lost acclimatization to temperature more quickly than did inbred males. B/K males gained acclimatization to temperature more quickly than any other group, but the K inbred males gained acclimatization more quickly than did either the K/B males or the B males. In all cases acclimatization is gained more quickly than it is lost.

3. The extent of acclimatization to temperature, as measured by the difference in survival times of 15°C. and 25°C. acclimatized flies in a range of lethal temperatures, was not found to be different in inbreds and hybrids.

4. The results suggest that hybrids can produce the enzymes necessary for acclimatization to temperature more rapidly than inbreds and confirms the hypothesis that hybrids are biochemically more versatile than inbreds.

5. The difference between the rates of gain and loss of acclimatization to temperature suggests that the processes involved in the enzyme changes are temperature dependent.

6. The absence of a difference in the extent of acclimatization to temperature

indicates that both inbred and hybrid *D. subobscura* are capable of producing those enzymes necessary for temperature acclimatization.

7. The high values of the temperature coefficients for heat death indicate that this process involves protein (enzyme) denaturation.

8. An ageing effect was observed in inbred flies.

This work was supported by a grant to one of us (M. J. H.) from the Agricultural Research Council. We are very grateful to Mr M. P. Curwen for advice and help on the statistical treatment of the data.

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