

## **'Heterosis' in $F_1$ mice in a cold environment**

BY S. A. BARNETT AND ELIZABETH M. COLEMAN

*Department of Zoology, The University, Glasgow*

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### 1. INTRODUCTION

The terms 'hybrid vigour' and 'heterosis' refer to the fact that outbred organisms are often more successful, on some measurable criterion, than inbred individuals of the same species. The criteria usually employed are measures of fertility or growth, but resistance to disease and yet others may be used. A current explanation of heterosis is in terms of the effects on ontogeny of gene interaction. It is assumed that there is an optimal direction and rate for each developmental process, at least for a given environment, and that the optimum course is followed more closely by heterozygous than by homozygous individuals (Waddington, 1942; Haldane, 1949, 1954; Lerner, 1954).

If heterosis is due to a 'buffering' action of the genes in ontogeny, it should follow that it would be more evident in an environment which, while permitting survival and reproduction, departs substantially from the optimum: in such an environment the effect of heterozygous loci ought to be magnified. This has been tested, in the work described below, on inbred and  $F_1$  mice reared in two environments. It had already been found that mice of strains A, A2G and C57BL could be maintained as permanent breeding stocks in an environment kept at  $-3^\circ\text{C}$ . (Barnett & Manly, 1954, 1959). Fertility is lower in these conditions than at  $21^\circ\text{C}$ ., especially in strain C57BL. The account that follows shows that the  $F_1$  mice obtained by crossing A2G and C57BL mice are more fertile than either parent strain at each temperature, but that the difference is much greater at  $-3^\circ\text{C}$ .; and  $F_1$  variation in certain parameters is lower at both temperatures but especially at  $-3^\circ\text{C}$ .

### 2. METHOD

The parental mice were of the A2G and C57BL strains, rigorously inbred. A breeding stock of each strain is maintained in each of two constant-temperature rooms, kept respectively at about  $21^\circ\text{C}$  and about  $-3^\circ\text{C}$ . They are fed on diet 41, and have cotton wool for nesting. The breeding performance of these mice, and the conditions in which they are kept, have been fully described elsewhere (Barnett, 1956; Barnett & Manly, 1956, 1959).

To provide the  $F_1$  mice in the cold room, fourteen pairs were mated; in each, the female was of the C57BL strain and the male A2G. The age at mating was about 6 weeks. Only five pairs produced young within 8 weeks of mating. In three of these pairs the male was of the fifth generation in the cold and the female of the

Table 1. *Breeding performance*

	21° C.			-3° C.		
	A2G	C57BL	F <sub>1</sub>	A2G	C57BL	F <sub>1</sub>
No. of pairs	15	15	11	19	10	12
Litters born per pair	5.1 ± 0.56	5.1 ± 0.27	4.6 ± 0.23	2.8 ± 0.20	2.4 ± 0.58	3.1 ± 0.08
Litters weaned per pair	4.5 ± 0.52	3.7 ± 0.40	4.5 ± 0.27	1.3 ± 0.19	1.1 ± 0.55	3.0 ± 0.12
Young born per pair	27.1 ± 3.06	33.5 ± 2.85	43.8 ± 0.99	10.9 ± 1.09	12.3 ± 3.16	27.7 ± 1.09
Young weaned per pair	20.7 ± 2.59	20.4 ± 3.46	39.1 ± 1.82	4.3 ± 0.73	5.3 ± 1.59	22.7 ± 1.51
No. of young born per litter	5.3 ± 0.20	6.5 ± 0.28	9.5 ± 0.40	4.1 ± 0.24	5.1 ± 0.48	9.0 ± 0.71
No. of young weaned per weaned litter	4.7 ± 0.22	5.6 ± 0.30	8.8 ± 0.37	3.2 ± 0.28	4.8 ± 0.59	7.6 ± 0.30

The standard error is given with each mean.

first generation: that is, the female's parents had been transferred to the cold before mating. In the other two pairs the male was of the eighth generation in the cold, the female of the second. In the warm room, the corresponding group consisted of five similar pairs, all of which produced young. The  $F_1$  young of these pairs, in both temperatures, were weaned at 3 weeks. Some were mated, each to a member of the same litter, at 5 weeks; and their breeding performance was recorded until they reached 28 weeks. The young of these pairs were weighed to the nearest 0.5 g. at the age of 3 weeks.

Others of the  $F_1$  young in the warm room were 'stressed' by transfer to the cold room at 22 days. Each mouse was placed alone in a cage containing cotton wool. As a control a litter-mate of the same sex was put in the same conditions in the warm room. Mortality and growth in these mice were recorded to the age of 6 weeks, when all survivors were killed. Comparison was made with mice of the parent strains similarly treated. The effect of cold stress on young inbred mice has been more fully described by Barnett, Coleman & Manly (1960).

### 3. RESULTS

#### (a) Breeding performance

The data on the breeding of the three kinds of pairs in each temperature are given in Table 1. The performance of the two parent strains has already been discussed by Barnett & Manly (1959).

At 21° C., as expected, the  $F_1$  pairs did better than either parent strain. The superiority of the  $F_1$  pairs appeared in the number of young per litter, both at birth and at weaning. There were consequently more  $F_1$  young, both born and weaned, per pair than C57BL or A2G. The superiority of the  $F_1$  strains was especially marked at weaning, since their losses between birth and 3 weeks were

Table 2. Percentage loss of young between birth and weaning

	21° C.	-3° C.
A2G	23.6	40.6
C57BL	39.1	56.9
$F_1$	10.7	18.1

See Table 1 for numbers of pairs and of young.

much lower than were those of the parent strains (Table 2): the number of young weaned per pair was 1.9 times the mean of the inbred strains.

At -3° C. the breeding of the  $F_1$  mice was again better than that of the parent strains at the same temperature, but the difference was much greater than that in the warm. This was shown in the number of litters born, and still more in the number weaned. Among the inbred mice losses of whole litters were common at -3° C. (Barnett & Manly, 1959), but out of the thirty-seven  $F_1$  litters only one

such loss occurred. Litter size, both at birth and at weaning, was about twice that of the parent strains. Consequently, the number of young born per pair was more than twice that in the inbred stocks, and the number weaned per pair was 4.7 times the mean of the parent strains.

The number of young weaned per pair is, of all the parameters measured, the most significant both from a biological point of view (that is, for fitness) and from the standpoint of a stockbreeder. Mortality after 3 weeks was negligible; consequently each mouse reared to that age was a potential breeder or, if kept but not mated, had a very high probability of becoming an adult. From this point of view there was, as would be expected, a marked advantage in hybridity at the (near optimum) temperature of 21° C., but a much greater advantage at -3° C.

The preceding paragraph requires qualification. Not all the mice that reached 3 weeks were fecund. Barnett & Manly (1959) had three barren C57BL pairs out of ten at -3° C., and other pairs have proved to be barren in subsequent breeding (unpublished). There was a smaller incidence of infecundity among the A2G mice. But, of the eleven F<sub>1</sub> pairs at -3° C., all were fertile and (as further discussed below) the degree of fertility varied little among the pairs. It follows that, of the young weaned by inbred pairs at -3° C., a substantial proportion would be unable to contribute to the next generation; while, of the young weaned by F<sub>1</sub> pairs, all (or nearly all) could be expected to be able to do so. The available data do not allow an exact estimate of the number of fertile young produced per pair, but it can be concluded that the figure for F<sub>1</sub> pairs in the cold would be more than 4.7 times the mean of the parent strains.

A further qualification concerns the interpretation of the figures for number of litters and of young born and weaned per pair (but not per litter). In these experiments breeding was recorded up to a given age. Consequently, the age at which the breeding began was a factor in reproductive performance. Table 3 shows the mean

Table 3. *Age in days of females at birth of first litter*

		A2G	C57BL	F <sub>1</sub>
21° C.	Range	61-99	59-131	52-66
	Mean with standard error	73.6 ± 3.09	79.4 ± 4.80	59.0 ± 1.15
-3° C.	Range	79-210	97-183	62-82
	Mean with standard error	117.1 ± 9.85	118.0 ± 10.39	72.4 ± 1.92

The numbers of females are the same as the numbers of pairs in Table 1.

age at first litter for each class of female. In both temperatures the F<sub>1</sub> mice began earlier than the inbred mice at the same temperature; further, their range was much smaller and (as discussed further in §3(d) below) variation was lower. Thus

the superior performance of the  $F_1$  mice, especially in the cold, was partly a consequence of their earlier maturity. Delay in the attainment of maturity, shown by Barnett & Coleman (1960) to occur in A2G females in the cold, was evidently not so great in the  $F_1$  mice.

Table 4. *Mass of young weaned per pair; numbers of pairs, and means with standard errors*

		21° C.	-3° C.
A2G	No. of pairs	12	13
	Mass weaned per pair (g.)	240.9 ± 39.08	44.4 ± 12.41
C57BL	No. of pairs	14	5
	Mass weaned per pair (g.)	189.2 ± 25.66	56.3 ± 23.42
$F_1$	No. of pairs	11	12
	Mass weaned per pair (g.)	350.5 ± 5.28	193.4 ± 12.05

The number of pairs in some classes is smaller than that in Table 1, since in some instances not all the young were weighed at 3 weeks.

Table 5. *Mean weights, with standard errors, of young aged 3 weeks, of inbred and  $F_1$  pairs*

		21° C.		-3° C.	
		♂	♀	♂	♀
C57BL	No.	88	96	18	19
	g.	8.4 ± 0.15	8.2 ± 0.14	7.4 ± 0.27	7.1 ± 0.23
A2G	No.	107	118	55	56
	g.	10.2 ± 0.15	9.8 ± 0.13	8.5 ± 0.17	8.3 ± 0.16
$F_1$	No.	225	242	122	151
	g.	9.2 ± 0.09	8.7 ± 0.08	8.5 ± 0.08	8.5 ± 0.07

(b) *Body weights of young at three weeks*

The preceding account has expressed the performance of breeding pairs in terms of the *numbers* of young born and weaned. A complete picture of the results of breeding must also include a statement of the *mass* of the young produced. Table 4 shows that at 21° C., in terms of total mass of 3-week young per pair, the  $F_1$  mice

do about 1.6 times as well as the mean of the parent strains. At  $-3^{\circ}\text{C}$ . the corresponding figure is 3.8. From this point of view the advantage of hybridity is twice as great at  $-3^{\circ}\text{C}$ . as at  $21^{\circ}\text{C}$ .

These figures, taken by themselves, could be a result of the production of many small mice or fewer large ones. The fact that the litters of  $F_1$  pairs, both at birth and at weaning, were much larger than those of the parent strains suggests the

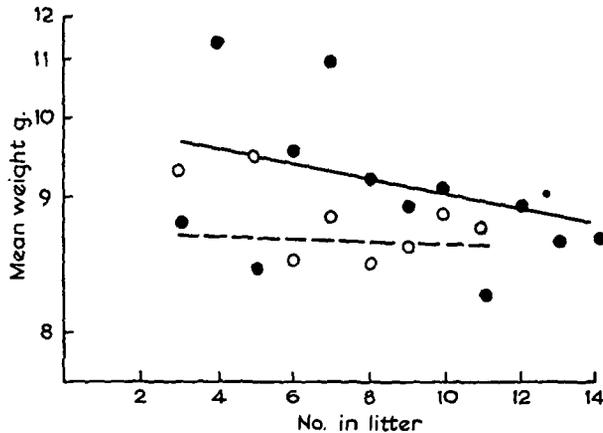


Fig. 1. The relationship between number in litter at 3 weeks and mean body weight (on a logarithmic scale) at that age:  $F_2$  males. The fitted regression lines are shown. Solid circles:  $21^{\circ}\text{C}$ .; open circles:  $-3^{\circ}\text{C}$ .

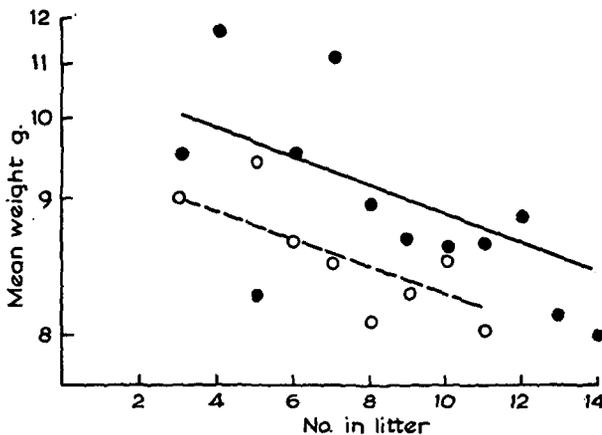


Fig. 2. As Fig. 1:  $F_2$  females.

possibility that the weights of the individual mice would be lower than those from the smaller inbred litters. (For references to the literature on the relationship of body weight to number in litter, see Barnett & Manly, 1959.) Table 5 shows that, despite the much larger litters of the  $F_1$  pairs, the mean weights at weaning of the young at  $21^{\circ}\text{C}$ . lay between those of the parent strains. At  $-3^{\circ}\text{C}$ . they were

almost identical with those of the A2G mice, and significantly higher than those of the C57BL mice.

Figs. 1 and 2 show the relation of log weight at 3 weeks to number in litter at that age. Especially for the females at both temperatures there is a clear indication of the expected decline in weight with increased number in litter (cf. Ashoub, Biggers, McLaren & Michie (1958), for a similar sex difference in TO mice). Nevertheless, even at  $-3^\circ\text{C}$ ., the figures show that members of litters of nine, ten and eleven still averaged above 8 g. at 3 weeks. The corresponding data for strains A2G and C57BL have been given by Barnett & Manly (1959), but with the sexes not separated. They found an indication of decline in body weight with litter size in strain C57BL at both temperatures, but in strain A2G only at  $-3^\circ\text{C}$ .

In general, then, while the number of hybrid mice produced is much higher than that of inbred mice, the body weights are nevertheless maintained at a level similar to that of the parent strains.

(c) Resistance of young to cold

Table 6 shows the mortality which resulted from transferring A2G and  $F_1$  mice at 22 days, and C57BL mice at 35 days, from  $21^\circ\text{C}$ . to  $-3^\circ\text{C}$ . (cf. Barnett &

Table 6. *Mice aged 3 weeks, reared at  $21^\circ\text{C}$ .: mortality during 7 days' exposure alone in a cage*

		Exposed at $-3^\circ\text{C}$ .		Controls at $21^\circ\text{C}$ .	
		♂	♀	♂	♀
A2G	No.	20	21	15	15
	% died	35.0	42.9	0.0	0.0
C57BL	No.	15*	14*	8	8
	% died	46.7	50.0	0.0	0.0
$F_1$	No.	24	24	20	24
	% died	12.5	8.3	0.0	4.2

\* Aged 5 weeks at transfer to  $-3^\circ\text{C}$ .

Manly, 1958; Barnett, Coleman & Manly, 1960). The hybrid young showed themselves more resistant to cold even than the A2G mice.

Fig. 3 (upper graphs) compares the growth of hybrid mice 'stressed' at 22 days with that of A2G mice similarly treated. There are no comparable figures for C57BL mice, since these were stressed only at the age of 5 weeks. Fig. 3 (lower graphs) gives similar data for the controls, including those of strain C57BL. Of the two parent strains the A2G mice were the heavier at ordinary temperatures.

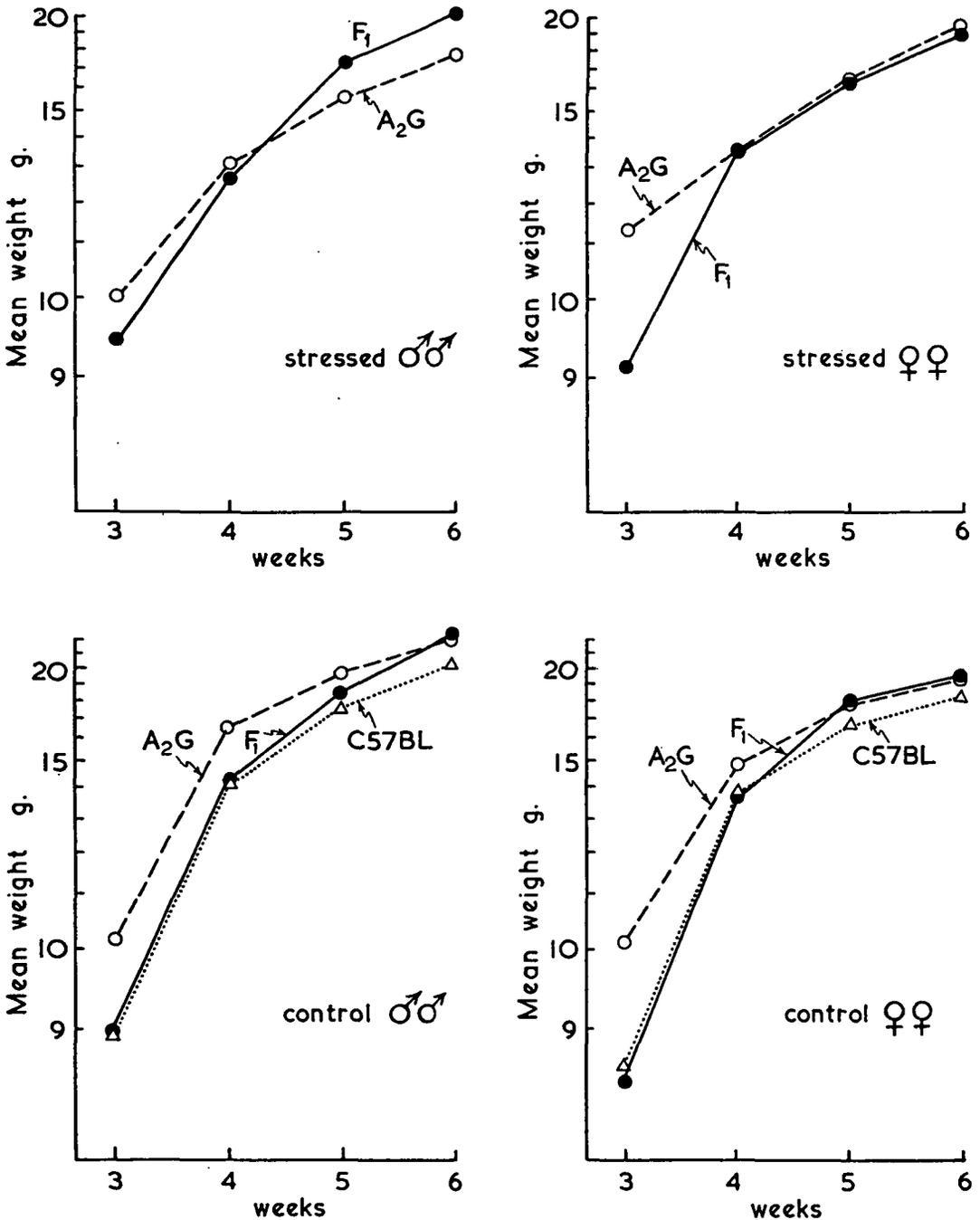


Fig. 3. Above: growth of F<sub>1</sub> and A<sub>2</sub>G mice after transfer to  $-3^{\circ}\text{C}$ . at the age of 22 days. Below: growth of F<sub>1</sub>, A<sub>2</sub>G and C57BL mice at  $21^{\circ}\text{C}$ . Males on left, females on right. The ordinates give weight on a logarithmic scale.

The stressed A2G mice of which the weights were recorded formed a biased sample, since they inevitably came from the two-thirds that survived the stress. By contrast, the  $F_1$  mice, with their lower mortality, were more nearly a representative sample. In each sex, and in each temperature, the  $F_1$  mice had a higher growth-rate. Since the mean weight of the  $F_1$  mice at 3 weeks was lower than that of this sample of A2G mice, the growth curves in each instance converge. By 6 weeks the stressed male  $F_1$  mice were heavier than the A2G males. In other classes the weights at 6 weeks of the A2G and  $F_1$  mice were almost identical. The control mice of the C57BL strain were lighter than the corresponding  $F_1$  mice at 6 weeks.

The superior performance of young  $F_1$  mice on being transferred to the cold is relevant to the greater fertility of the  $F_1$  mice bred in the cold. It has been shown above that the greater number of young they rear to weaning is partly a result of a much lower post-natal mortality. A high degree of resistance to cold is thus reflected in the observations on mortality both of  $F_2$  nestlings born in the cold and of stressed  $F_1$  mice.

(d) Variation

The preceding account has dealt with *average* performance in reproduction and resistance to cold. There remains the question of the extent to which *variation* in performance is influenced by hybridity (cf. Grüneberg, 1954; McLaren & Michie, 1954).

Table 7 gives the coefficients of variation for the parameters of reproduction, for each class of mice. As already reported by Barnett & Manly (1959), the mice

Table 7. Coefficients of variation, per cent

	A2G		C57BL		$F_1$	
	21° C.	-3° C.	21° C.	-3° C.	21° C.	-3° C.
Litters born per pair	41.0 (29.8)	29.7 (21.2)	19.7	64.1 (26.3)	16.6*	9.0*†
Litters weaned per pair	39.5 (35.5)	59.4 (39.0)	40.8	72.5 (46.4)	20.0	13.5†
Young born per pair	42.3 (31.9)	42.4 (35.1)	31.9	77.1 (34.1)	7.5*§	13.6†
Young weaned per pair	46.8 (41.2)	72.6 (53.4)	63.6	90.0 (51.8)	15.5	23.0
Young born per litter	33.1	40.5	37.6	46.1	30.0	48.2
Young weaned per weaned litter	38.0	41.7	39.4	35.2	29.3	23.4

The figures in brackets are the coefficients calculated only for those pairs whose record was above zero.

- \* Variance significantly different from A2G ( $P < 0.05$ )
- † " " " " C57BL ( $P < 0.01$ )
- ‡ " " " " " ( $P < 0.1$ )
- § " " " " " ( $P < 0.05$ )

In each instance comparison was made between  $F_1$  and inbred mice in the same temperature.

of the inbred strains tend to vary more at  $-3^{\circ}\text{C}$ . than at  $21^{\circ}\text{C}$ . The effect of hybridity was to reduce variation in most of the parameters of reproduction, at both temperatures. The  $F_1$  mice showed greater uniformity in the number of litters born per pair, even though their average performance in this respect differed little from that of the parent strains. In litters weaned per pair the effect of environmental temperature on variation was very marked: the low temperature did not increase variation in the  $F_1$  mice, but greatly increased it in both parent strains if pairs which weaned no young are included. In number of young born and number of young weaned per pair, the  $F_1$  mice showed greater uniformity at both temperatures. There was no significant effect of temperature on variation in numbers of young born or weaned *per litter*.

#### 4. DISCUSSION

Heterosis may be defined as the occurrence of greater fitness in outbred than in inbred individuals of the same species. 'Fitness' is used in a Darwinian sense; its meaning has been fully discussed by Thoday (1953). As indices of fitness it is reasonable to use such parameters as the number of young weaned per pair or resistance to disease, but not size or growth rate: there is no *a priori* likelihood that more rapid growth or greater adult body weight will be advantageous. The fact that outbreeding does sometimes lead to increased growth has led Dobzhansky (1950) to propose 'luxuriance' as a name for this phenomenon, in order to distinguish it from heterosis as defined above.

'Inbreeding depression' in domestic mammals in respect of fertility and of viability of the young is well known (Donald, 1955). This is the inverse of heterosis. Gates (1925) and Chai (1956) have recorded examples of higher fertility in outbred mice. Marshak (1936) and Falconer & King (1949) have also observed luxuriance, or 'weight heterosis', in  $F_1$  mice. Butler (1958) found that  $F_1$  mice from strains BALB and C57BL had larger litters than the parent strains. The data from our control mice illustrate the superior breeding performance of  $F_1$  mice in near-optimum conditions. They also give some evidence on the extent to which different stages in the production of young were influenced by hybridity. The number of *litters* born to  $F_1$  pairs in the warm room was not increased; nor was the number of litters weaned by them significantly different from that of the inbred pairs. But the numbers of *young* born and weaned were greatly increased. Three groups of factors were no doubt involved: (i) conditions in the uterus; (ii) conditions in the nest, including milk supply; (iii) the genetical character of the  $F_2$  young. (i) and (ii) have been discussed by Barnett & Manly (1959) in relation to reproduction in the inbred stocks. The data on the  $F_1$  mice do not allow us to distinguish with certainty the effects of these influences from those of (iii). However, the facts that (a) the number weaned per litter by the  $F_1$  mice was nearly twice that of inbred strains and (b) the  $F_2$  mice at weaning were intermediate in weight between the parent strains show that the  $F_1$  females must have had a higher milk yield than the inbred females.

The main interest of our breeding data is in the effect of hybridity in the cold environment. Here the superiority of the  $F_1$  mice was far more marked. The number of young born per pair was more than twice the mean of the inbred strains, and presumably reflects a better uterine environment. Still more impressive was the nearly fourfold increase in the number of young weaned per pair. As in the warm environment, this presumably represented the combined effects of maternal efficiency and the 'vigour' of the  $F_2$  young. The superior vigour, in the sense of resistance to cold, of young  $F_1$  mice reared in the warm was clearly shown in the results of subjecting them to cold stress at weaning.

An important question is to what extent the performance of the  $F_1$  mice in the cold environment was more uniform, as well as more successful, than that of the parent strains. Table 7 shows that percentage coefficients of variation for certain aspects of reproductive performance in the  $F_1$  mice were much lower than in the inbred mice. This held especially for the numbers of young born and weaned per pair in both temperatures. There was also a marked regularity in number of litters born per pair in the  $F_1$  mice at  $-3^\circ\text{C}$ .

The coefficients of variation for the  $F_1$  mice were low in both temperatures. Except in the number of young *born per litter*, the pattern of increased variation in the cold, which was especially shown by the C57BL mice, did not appear in the hybrids. In the  $F_1$  mice the coefficients for numbers of young born and weaned per pair were higher in the cold than in the warm, but variation in these parameters was in any case very low in both environments.

The comments above apply to comparisons of variation estimated for all the pairs in each class. However, some inbred pairs were barren, namely, one A2G pair in each temperature and three C57BL pairs at  $-3^\circ\text{C}$ ; further, one fecund A2G pair at  $-3^\circ\text{C}$  reared no young to 3 weeks (Barnett & Manly, 1959). In Table 7 the figures in brackets give the coefficients of variation calculated with these 'zeros' omitted; they show that, especially in the C57BL mice in the cold, the zeros made an important contribution to the amount of variation. Nevertheless, even with the coefficients calculated in this way, the general picture remains unchanged.

Ashoub, Biggers, McLaren & Michie (1958) have given an example of increased variation in body weight in a heterogeneous stock of TO mice in unfavourable environments: they subjected pregnant females and their young to temperatures of  $5^\circ\text{C}$  or  $28^\circ\text{C}$ ; controls were, like ours, kept at  $21^\circ\text{C}$ . The young born to the females were weighed at 1, 2, 3 and 4 weeks, and it was the variation in these weights that was found to be increased by the extreme temperatures. By contrast, our mice weighed at 3 weeks (and, in the case of the 'stressed' mice, also at 4, 5, and 6 weeks) showed no significant effect of temperature on variation in body weight. This is in conformity with the fact that the mean weights of the different classes of mice were similar at each age. By contrast, the means in the parameters of reproduction differed widely, and it was here that differences in variance were found. The main effect of the cold on the young inbred mice of our experiments was to kill them, and the dead mice represent the extreme variants. The difference

between our young mice and those of Ashoub and his colleagues may be due either to their use of a different stock or to the higher temperature to which their 'cold-stressed' mice were subjected.

Our  $F_1$  mice, then, showed three major effects of hybridity: (i) heterosis in reproductive performance in both environments; (ii) a greater advantage of hybridity in the less favourable environment; (iii) a greater phenotypic uniformity, especially in the less favourable environment.

From a stockbreeding point of view the value of using hybrid animals was more marked when the animals were in inferior conditions, whether value is measured in terms of numbers or of biomass. This economic attitude to hybrid vigour is, however, superficial. What is the significance of heterosis in terms of the natural-selection process and the genetical mechanism? Discussions of this question have been published by Haldane (1949, 1955), Lerner (1954), Dobzhansky & Levene (1955), Jinks & Mather (1955), McLaren & Michie (1956) and Maynard Smith (1956), and several hypotheses have been advanced to account for the connexion between heterosis and adaptability. Low variance in a given environment and the ability to respond adaptively to a changed environment have been held to be aspects of a single type of process, that is, they have been supposed to reflect an increase in the resources of the genotype: this is thought to confer both improved 'homeorhesis' (Waddington, 1957) in a given environment and also the possibility of alternative developmental paths in different environments. However, heterozygosis does not always produce developmental stability (e.g. Tebb & Thoday, 1954; Jinks & Mather, 1955). It has therefore been suggested—for example, by Jinks & Mather—that superior fitness of heterozygotes, when this is in fact observed, must be a result of natural selection, and need not be attributed to any inherent property of the heterozygous state as such. However, the two hypotheses are not necessarily opposed: it remains possible that the nature of gene action is such that heterozygotes must often tend to be more versatile in their response to widely different environments than homozygotes; yet the extent to which this is true, and the precise form of the heterosis, may be influenced by natural selection.

The data given in this paper support the belief that heterozygosis confers on an organism an increased ability to withstand the effects of environmental hazards on its development; in this regard the marked uniformity of the  $F_1$  mice in the unfavourable environment is especially important. They also suggest a possibility for further study. If heterosis is a result of the action of natural selection on heterozygotes, the more important a process is for physiological adaptation to environmental hazards, the more it should be enhanced by hybridity. This possibility is being studied.

##### 5. SUMMARY

The effect of hybridity on reproduction in mice has been studied in two environmental temperatures: 21° C. ('normal') and -3° C. (unfavourable). Mice of inbred strains A2G and C57BL are maintained as permanent breeding stocks in each of the two temperatures. In each temperature the two strains were crossed,

and the reproduction of  $F_1$  pairs, to the age of 28 weeks, compared with that of the parent strains.

At 21° C. the expected superior fertility of the  $F_1$  mice was found: the number of litters produced was not affected, but there were more young produced per pair. The number of young per pair reared to 3 weeks was about twice the mean of the parent strains at the same temperature.

At -3° C. the difference was greater. The  $F_1$  pairs produced more and larger litters than the parent strains, and deaths between birth and weaning at 3 weeks were few. The number of young per pair reared to 3 weeks was nearly five times the mean of the parent strains. Part of the superiority of the  $F_1$  mice, at both temperatures, was due to the fact that they began to breed earlier.

Despite the larger litters produced by the  $F_1$  pairs, at 21° C. the mean weight of the  $F_2$  young at the age of 3 weeks was intermediate between those of the parent strains. At -3° C. it was the same as that of the heavier parent strain, namely, A2G.

As a further test of resistance to cold,  $F_1$  mice born in the warm environment were transferred to the cold at the age of 22 days and there placed each alone in a cage, with nesting material. In these conditions they had a higher survival rate than the young of either of the parent strains. They also grew faster than the A2G mice.

The  $F_1$  mice were not only more fertile than the inbred mice, but also more uniform in breeding performance. This difference was especially marked in the less favourable environment.

These observations are in conformity with the view that heterosis is a consequence of heterozygosis; and that it depends on an enhanced ability to withstand disturbances of developmental and physiological equilibria.

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