

Hybrids show parental influence in the adaptation of wild house mice to cold

S. A. BARNETT AND R. G. DICKSON

Department of Zoology, Australian National University*

(Received 25 May 1986 and in revised form 5 June 1987)

Summary

Wild house mice, *Mus musculus*, were bred (a) at 3 °C ('Eskimo') and (b) at 23 °C. Mice of the ninth generation bred at 23 °C were transferred to the cold environment. Their young, and Eskimo of the same (tenth) generation, were mated to give the four possible types of pairs: controls; the two reciprocal hybrid pairings; and Eskimo. In the resulting (eleventh) generation there were therefore two hybrid classes, genetically identical but with different parentage. The growth and reproduction of the eleventh generation were studied. At all ages from birth, mice with Eskimo mothers were heavier than those with control mothers. They were also better breeders: (1) they matured earlier; (2) their litters were larger; (3) the mortality of their young in the nest was lower. In one feature there was heterosis: of the four classes, the hybrids with Eskimo mothers produced the largest litters. These and previous findings suggest rapid selection, in the cold, for changes in growth, reproductive physiology and other aspects of metabolism. The cold-adapted mice of a given generation differed from the controls partly as a result of favourable parental effects, which acted in conjunction with genetical differences. It is hypothesized that the ecological versatility of *Mus musculus* depends partly on the presence, in each population, of alternative genotypes which allow rapid adaptation to new conditions.

1. Introduction

Wild house mice, *Mus musculus*, can breed and multiply in environments below 0 °C, although their adult body weight is usually less than 20 g (reviewed by Barnett, 1973; Berry, 1981; Bronson, 1979; Jakobson, 1981). This ability raises questions concerning not only adaptation to cold but also the interactions of genotype, parental effects and environment.

During ontogenetic cold-adaptation, the metabolic rate (resting oxygen consumption) of a mouse is raised (Barnett, Coleman & Manly, 1959), and there are corresponding changes in many organs; these changes are enhanced during pregnancy and lactation (Barnett & Widdowson, 1971). In a population transferred to a cold environment, genetical divergence from the parent population must be expected, and the result may be phenotypic differences which enhance fitness in the cold. Both types of cold adaptation may entail changes in parental, especially maternal, performance which influence the fitness of the next generation.

Here we are concerned with such maternal effects. To distinguish them from the direct action of genetical differences, we crossed wild-type mice from two laboratory colonies, one of which had been kept for nine generations in a cold environment ('Eskimo'), the other in a warm one. The Eskimo mice had, over ten generations, become progressively heavier and more fertile (Barnett & Dickson, 1984*a, b*). We compare below the genetically identical offspring from reciprocal crosses; of these one class had Eskimo mothers, the other control mothers. In an earlier study, in which cross-fostering was used, we found favourable effects of having an Eskimo foster mother, but these were clearly evident only in the cold environment (Barnett & Dickson, 1986). The present experiments were therefore carried out only in the cold: all mice studied were conceived, born and reared in the cold environment. The intention was to reveal more fully the scope of maternal influence in the cold-adaptation of mice.

2. Method

House mice were trapped on poultry farms near Canberra, and mated. Their offspring were used to

* Address for reprints: R. G. Dickson, CSIRO Wildlife Research, Box 84, P.O., Lyneham, A.C.T., 2602, Australia.

Table 1. *The mice. All, except the controls of generation 9, were conceived and reared in the cold environment*

Generation	Pairs			
9	Control ♂ ^a —	—	—	Eskimo ♂
	Control ♀ ^a —	—	—	Eskimo ♀
10	Control ♂	Eskimo ♂	Control ♂	Eskimo ♂
	Control ♀	Control ♀	Eskimo ♀	Eskimo ♀
11	Control ♂	Hybrid with	Hybrid with	Eskimo ♂
	Control ♀	control mother	Eskimo mother	Eskimo ♀

Generation 12 was represented, in the present experiments, only by the young of the two types of hybrid (Table 6).

^a Transferred from the warm environment at mating.

found two closed colonies, one at an ambient temperature of 3 °C, the other at 23 °C. In each generation there were at least 15 permanently mated pairs, of which the members were never siblings. The mice were kept in black plastic cages with wire lids. Methods of handling are described by Evans, Smart & Stoddart (1968). Water and Mecon rat cubes were always available. At the ninth generation, 15 pairs of mice were transferred from the warm environment to the cold at mating. Their young, and those of ninth-generation Eskimo, were used to provide 15 pairs of each of four classes: controls; the two reciprocal hybrid classes; and Eskimo (Table 1). These made the tenth generation, and their offspring the eleventh generation.

The growth and reproduction of the mice of the eleventh generation were recorded. Infant mice were weighed to 0.01 g, on a direct-reading balance, at 0, 10 and 21 days. For analysis, the weights of males and females were pooled, and litters, not individuals, were

treated as replicates. Thereafter, some males were kept in groups of three and weighed at 16 weeks. Other males were mated, and weighed to 0.1 g when killed at 30–33 weeks. Females, after three weeks, were weighed only at the time of mating. After the age of three weeks individual weights were treated as replicates. For observation of reproductive performance, mice were mated at 8–10 weeks. Pairs were kept for 23 weeks from mating, or until their last litter, born in that period, was weaned. Young mice were taken from their parents at 21 days.

Comparisons of means are by Student's *t* test, two-tailed. For some findings we also use two-way analysis of variance, with replication.

3. Results

(i) Growth

As expected, the Eskimo mice were heavier than the mice of the other three classes, even the hybrids with

Table 2. *Body weights (g) of generation 11. Means with their standard errors. (Figures in parentheses give number of litters for 0–3 weeks, numbers of mice thereafter.)*

Age (weeks)	Controls	Hybrids		Eskimo
		with control mothers	with Eskimo mothers	
Both sexes				
0	1.29 ± 0.02 (26)	1.30 ± 0.02 (29)	1.45 ± 0.02** (33)	1.47 ± 0.02 (36)
1.5	4.3 ± 0.1 (28)	4.3 ± 0.1 (43)	5.3 ± 0.1** (31)	5.4 ± 0.1 (46)
3	7.3 ± 0.1 (24)	7.2 ± 0.1 (38)	8.4 ± 0.2** (31)	8.6 ± 0.1 (47)
Females				
8–10	11.8 ± 0.6 (10)	13.9 ± 0.4 (10)	14.9 ± 0.4* (10)	16.4 ± 0.6 (10)
Males				
16	18.5 ± 0.3 (40)	19.3 ± 0.3 (55)	22.0 ± 0.3** (44)	23.9 ± 0.4 (49)
30–33	23.2 ± 1.0 (19)	22.0 ± 0.9 (10)	23.4 ± 0.6* (11)	28.1 ± 1.3 (9)

Difference from hybrids with control mothers: **P* < 0.01; ***P* < 0.001. (Values of *P* are given only for differences between the two classes of hybrids.)

Table 3. Sizes at birth of litters born to parental (tenth) generation. Means with their standard errors

	Mothers	
	Control	Eskimo
Fathers		
Control	5.8 ± 0.3	6.5 ± 0.2
Eskimo	6.9 ± 0.3	6.1 ± 0.3

Table 4. Reproductive performance, recorded for 26 weeks from pairing, of 11th generation. Means with their standard errors

	Controls	Hybrids		
		with control mothers	with Eskimo mothers	Eskimo
No. of pairs	10	8	10	9
Pairing to first litter, days	65.4 ± 15.4	72.0 ± 6.4	40.2 ± 10.5*	34.1 ± 3.6
Litter/pairs	3.6 ± 0.5	3.5 ± 0.3	5.0 ± 0.5*	5.1 ± 0.3
Young born/pair	18.5 ± 3.9	20.1 ± 2.1	38.9 ± 5.2**	30.3 ± 4.1
Young weaned/pair	14.3 ± 4.3	11.0 ± 1.6	33.6 ± 5.4**	21.1 ± 6.0

Difference from hybrids with control mothers: * $P < 0.05$; ** $P < 0.01$. (For a full account of the differences between Eskimo and controls, see Barnett & Dickson, 1984a).

Table 5. Sizes of litters born to 11th generation females. Means with their standard errors, and analysis of variance

	Control mother	Eskimo mother
Purebred	5.2 ± 0.4	5.9 ± 0.6
Hybrid	5.8 ± 0.3	7.7 ± 0.3 ^a

Source of variation	D.S.	Mean square	F	P
Purebred type	1	8.0	1.5	>0.2
Hybrid type	1	56.1	10.8	<0.01
Interaction	1	16.5	3.2	>0.05
Residual	122	5.2		

^a Different from other classes ($P < 0.01$).

Table 6. Body weights (g) of offspring of hybrids. Means with their standard errors. (Numbers of litters in parentheses)

Age (days)	Parents	
	Hybrids with control mothers	Hybrids with Eskimo mothers
0	1.49 ± 0.03 (18)	1.43 ± 0.02 (38)
10	5.0 ± 0.16 (18)	5.3 ± 0.10 (38)
21	8.6 ± 0.25 (17)	8.7 ± 0.13 (37)

Eskimo mothers; and the controls were the lightest class (Table 2). For each sex and age, weights tend to rise as one reads from left to right across the table. Comparison of the two hybrid classes shows a maternal effect: hybrids with Eskimo mothers were consistently heavier than those with control mothers. The influence of the mother, however, diminished with age: at 30 weeks, hybrid males with Eskimo mothers had grown little since 16 weeks, and were hardly heavier than those with control mothers; in the

same period, the weight of the pure Eskimo males rose by about 17%.

The superior body weights of the young of Eskimo females were not due to the production of smaller litters: on the contrary, the controls, which were lightest at birth, came also from the smallest litters (Table 3). There was, however, a relationship of survival with litter size. As described elsewhere (Barnett & Dickson, 1984a), mortality, during the first three weeks, within surviving litters was low; but there was a substantial loss of whole litters. For all eight classes pooled, the mean size at birth of surviving litters was 7.1 ± 1.2 ($N = 251$); the corresponding figures for litters all of whose members died before 3 weeks were 4.5 ± 1.2 ($N = 117$; $P < 0.01$).

(ii) Reproduction

In reproductive performance the hybrids with control mothers resembled the pure controls and those with Eskimo mothers either resembled the pure Eskimo or surpassed them (Table 4). The hybrids with Eskimo mothers were superior in three main ways. (1) The first litter was born earlier; hence there were more litters to a pair. (2) The litters themselves were larger than those of any other class (Table 5); hence the mean number of young born to a pair was nearly twice that of hybrids with control mothers. (3) The figures of mortality between birth and 21 days were 14% and 45%, respectively. As a result, the hybrids

with Eskimo mothers weaned nearly three times as many young as did those with control mothers.

There was also an instance of heterosis: the litters of the hybrids with Eskimo mothers were, for wild mice, exceptionally large. Variance in litter size at birth was predominantly due to the contribution of the type of hybrid (Table 5). Large litters might be expected to result in smaller young. We therefore compare, in Table 6, the body weights of the offspring of the two hybrid types. These young, members of the twelfth generation, were virtually equal in weight at 0–21 days. The exceptionally large litters reared by hybrids with Eskimo mothers were therefore not produced at the expense of the growth of the young.

4. Discussion

The investigation of parental influences presents severe problems of controls. Three are relevant here. (1) Sex linkage can produce differences between reciprocal hybrids. In our experiments, however, the effects of parentage were shown by both sexes, and so sex linkage cannot explain them. (2) Our experiments did not control for paternal effects: hybrids with control mothers had Eskimo fathers and conversely. Eskimo males improve the chances of survival of young in the nest, probably by giving them more care than is provided by other males (Barnett & Dickson, 1985). Any such effect would, however, tend only to reduce the differences between the two hybrid classes; hence our conclusions on the character of the maternal influences we observed are unaffected. (3) The differences between Eskimo and control females might be a result not of a genetical change but of physiological adaptation by the Eskimo mice. The controls of generations 10 and 11 were, however, themselves physiologically cold-adapted: they had been both conceived and reared in the cold. The superiority of the Eskimo mice over the controls has also been shown in previous experiments, in which Eskimo mice were returned to the warm environment: there they exceeded the indigenous mice in growth and reproductive performance (Barnett & Dickson, 1984*a*; Barnett *et al.* 1975). These findings provide the best index of the genetical divergence between the two stocks.

The primary influence of mothers on their young is on their survival and growth *in utero* and in the nest. Conformably, hybrids with Eskimo mothers were heavier than those with control mothers (Table 2). Eskimo females secrete more milk than do control females, and their milk is more calorogenic (Barnett & Dickson, 1984*a*). To this evidence of the superior post-natal environment provided by Eskimo females the present findings add evidence on the uterine environment: body weights at birth (Table 2) indicate a higher growth rate in an Eskimo uterus.

To interpret body weights one must allow for the effect of litter size. The simplest assumption is of a

negative correlation between body weight and the number of young with which the mouse has been gestated and reared. This is sometimes taken as a universal rule (e.g. Drickamer, 1976; Eisen & Roberts, 1981; and references therein); but in fact it often does not fit findings on laboratory mice (reviewed by Barnett, 1973): and in an earlier study of wild house mice in Scotland no simple relationship was found (Barnett *et al.* 1975). Similarly, in the present study, there was no such relationship between classes of different mean weights: that is, classes with larger litters did not have lighter young: the superior weight of the young of the Eskimo females was not achieved by a reduction in litter size.

The absence of a decrement in litter size matches the finding, in the present experiments, that small litters are less viable than large ones. It also fits the positive correlation of nest temperature with the size of two-day-old litters found by Barnett & Dickson (1984*a*). Evidently, the maximum adaptive effect (resulting from enhanced thermal insulation) is conferred by a combination of superior body weight with a large litter.

Body weight is also important in the commonly found relationship of female body weight with reproductive performance, especially with (1) age at maturation and (2) the number and weight of the young. Domestic mice have their first oestrus when they reach a particular weight (Barnett & Coleman, 1959; Falconer, 1984); the age at which they do so varies with nutritional state, environmental temperature and other factors. In the present study the index of maturation was the interval between mating and the birth of the first litter; and, in accordance with expectation, the shortest intervals recorded were those of the two heaviest classes (Table 4).

The relationship of female body weight with the output of young has been recorded among domestic mice selected for large or small size. Body weight is positively correlated with ovulation rate (Brien, Sharp, Hill & Robertson, 1984; Elliott, Legates & Ulberg, 1968; Land, 1970) and hence with litter size at birth (reviewed by Pelikán, 1981). Cross-fostering between large and small females has revealed a strong maternal influence on early post-natal growth, attributed to differences in the amount of milk secreted (Al-Murrani & Roberts, 1978; Eisen & Roberts, 1981).

Our Eskimo mice were not systematically selected for any one feature such as size. In the early generations, however, many pairs were barren and infant mortality was high (Barnett & Dickson, 1984*a*). Hence, instead, they were subjected to a non-specific action of the cold environment on fertility. None the less, the Eskimo mice resembled in three respects the domestic mice selected for large size. (1) The large Eskimo litters probably reflected a high ovulation rate. (2) The early birth of the first litters of the Eskimo indicated early maturity. (3) There was also a similarity in milk secretion, for the milk supply of

Eskimo females is superior to that of controls in both quantity and quality (Barnett & Dickson, 1984*b*). The large domestic mice are, however, said only to produce *more* milk, whereas the distinctive feature of Eskimo milk is its high concentration of solids.

Superior growth rate is a consistent accompaniment of adaptation to cold in our experiments. In a previous study and in our present Eskimo population body weight rose progressively over generations in the cold. In a cold environment, other things being equal, a large mouse is better insulated than a small one. Our experiments could therefore be interpreted as an example of selection for an increase in weight or size. The changes in reproductive efficiency would then appear as by-products of a superior growth rate. It is, however, uncertain whether unusually large adult size endows a mouse with extra fitness. Free-living populations of house mice can be successful in exceptionally cold environments without any unusual body weight or shape (Berry, Jakobson & Triggs, 1973; Berry, Peters & van Aarde, 1978; Jakobson, 1981). The findings quoted above suggest that body weight early in life is a more significant factor.

A possible complication emerges from the work of Lynch & Roberts (1984). In their experiments the nests of domestic mice selected for large size provided better thermal insulation than those of mice selected for small size. Our wild mice learn to build better nests as part of their ontogenetic adaptation to cold (Wolf & Barnett, 1977); and the parental attention received by our Eskimo mice in the cold is superior to that received by controls in the same environment (Barnett & Foster, 1981; Barnett & Dickson, 1985). But we have no information on whether Eskimo mice build exceptional nests.

The 'syndrome' of changes undergone by our mice in the cold can now be summed up. Cross-fostering has provided some evidence of an intrinsically higher growth rate in the first weeks after birth (Barnett & Dickson, 1986); but the principal adaptive changes recorded concern parental, especially maternal influences. Eskimo females in the cold provide better environments for their young both before and after birth: these enhance growth and also (with paternal help in the nest) improve survival.

This is a result of 'selection' over only a few generations. The possibility of very rapid genetical change in rodent populations has been advanced by Chitty (1967, 1977) to account for the fluctuations in numbers of voles (*Microtus* spp.). His hypothesis presumes the presence, in each population, of rare genotypes which, in certain conditions, can quickly become common. The genetical variation is held to influence behaviour. The hypothesis has still to be fully tested, but some findings are in its favour (reviewed by Krebs, 1978; Tamarin, 1983). Perhaps, similarly, in each population of house mice there is a reservoir of genotypes which allow rapid adaptation, over a few generations, to new environmental demands.

References

- Al-Murrani, W. K. & Roberts, R. C. (1978). Maternal effects on body weight in mice selected for large and small size. *Genetical Research* **32**, 295–302.
- Barnett, S. A. (1973). Maternal processes in the cold-adaptation of mice. *Biological Reviews* **48**, 477–508.
- Barnett, S. A. & Coleman, E. M. (1959). The effect of low environmental temperature on the reproductive cycle of female mice. *Journal of Endocrinology* **19**, 232–240.
- Barnett, S. A., Coleman, E. M. & Manly, B. M. (1959). Oxygen consumption and body fat of mice living at -3°C . *Quarterly Journal of Experimental Physiology* **44**, 43–51.
- Barnett, S. A. & Dickson, R. G. (1984*a*). Changes among wild house mice (*Mus musculus*) bred for generations in a cold environment, and their evolutionary implications. *Journal of Zoology* **203**, 163–180.
- Barnett, S. A. & Dickson, R. G. (1984*b*). Milk production and consumption and growth of young of wild mice after ten generations in a cold environment. *Journal of Physiology* **346**, 409–417.
- Barnett, S. A. & Dickson, R. G. (1985). A paternal influence on survival of wild mice in the nest. *Nature, London* **317**, 617–618.
- Barnett, S. A. & Dickson, R. G. (1986). Interaction of genotype and parental environment in the adaptation of wild house mice to cold. *Journal of Zoology* **208**, 531–540.
- Barnett, S. A. & Foster, K. A. (1981). Cold adaptation and the parent–young interactions of wild house mice, *Mus musculus*. *Physiology and Behavior* **26**, 839–843.
- Barnett, S. A., Munro, K. M. H., Smart, J. L. & Stoddart, R. C. (1975). House mice bred for many generations in two environments. *Journal of Zoology* **177**, 153–169.
- Barnett, S. A. & Widdowson, E. M. (1971). Organ weights and body composition of parturient and lactating mice, and their young, at 21°C and -3°C . *Journal of Reproduction and Fertility* **26**, 39–57.
- Berry, R. J. (1981). Population dynamics of the house mouse. *Symposia of the Zoological Society of London* **47**, 395–425.
- Berry, R. J., Jakobson, M. E. & Triggs, G. S. (1973). Survival in wild-living mice. *Mammal Review* **3**, 46–57.
- Berry, R. J., Peters, J. & van Aarde, R. G. (1978). Sub-antarctic house mice: colonization, survival and selection. *Journal of Zoology* **184**, 127–141.
- Brien, F. D., Sharp, G. L., Hill, W. G. & Robertson, A. (1984). Effects of selection on growth, body composition and food intake in mice. II. Correlated responses in reproduction. *Genetical Research* **44**, 73–85.
- Bronson, F. H. (1979). The reproductive ecology of the house mouse. *Quarterly Review of Biology* **54**, 265–299.
- Chitty, D. (1967). The natural selection of self-regulatory behaviour in animal populations. *Proceedings of the Ecological Society of Australia* **2**, 51–78.
- Chitty, D. (1977). In *Evolutionary Ecology* (ed. B. Stonehouse and C. Perrins), pp. 27–32. Baltimore: University Park Press.
- Drickamer, L. C. (1976). Effect of litter size and sex ratio of litter on the sexual maturation of female mice. *Journal of Reproduction and Fertility* **46**, 369–374.
- Eisen, E. J. & Roberts, R. C. (1981). Postnatal maternal effects on growth and fat deposition in mice selected for large and small size. *Journal of Animal Science* **53**, 952–965.
- Elliott, D. S., Legates, D. E. & Ulberg, L. C. (1968). Changes in the reproductive processes of mice selected for large and small body size. *Journal of Reproduction and Fertility* **17**, 9–18.
- Evans, C. S., Smart, J. L. & Stoddart, R. C. (1968).

- Handling methods for wild house mice and wild rats. *Laboratory Animals* **2**, 29–34.
- Falconer, D. S. (1984). Weight and age at puberty in female and male mice of strains selected for large and small body size. *Genetical Research* **44**, 47–72.
- Jakobson, M. E. (1981). Physiological adaptability: the response of the house mouse to variations in the environment. *Symposia of the Zoological Society of London* **47**, 301–335.
- Krebs, C. J. (1978). A review of the Chitty hypothesis of population regulation. *Canadian Journal of Zoology* **56**, 2463–2480.
- Land, R. B. (1970). Genetic and phenotypic relationships between ovulation rate and body weight in the mouse. *Genetical Research* **15**, 171–182.
- Lynch, C. B. & Roberts, R. C. (1984). Aspects of temperature regulation in mice selected for large and small size. *Genetical Research* **43**, 299–306.
- Pelikán, J. (1981). Patterns of reproduction in the house mouse. *Symposia of the Zoological Society of London* **47**, 205–229.
- Tamarin, R. H. (1983). Animal population regulation through behavioral interactions. In *Advances in the Study of Mammalian Behavior* (ed. J. F. Eisenberg and D. G. Kleiman), pp. 698–720. Shippenberg, PA: American Society of Mammalogists.
- Wolfe, J. L. & Barnett, S. A. (1977). Effects of cold on nest-building by wild and domestic mice, *Mus musculus* L. *Biological Journal of the Linnean Society* **9**, 73–85.