Genet. Res., Camb. (1986), **48**, *pp.* 95–100 *With 2 text-figures Printed in Great Britain*

Heterosis among lines of mice selected for body weight

3. Thermoregulation

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(*Received 29 July 1985 and in revised form 7 May 1986*)

Summary

Crosses were made among 18 lines of mice, six previously selected for large 6-week weight, six for small 6-week weight, and six unselected controls, comprising a complete diallel cross among sizes and a partial diallel cross among replicate lines within sizes, and all purebred matings. Across all groups large size was associated with lower weight-specific food consumption and brown adipose tissue, and increased nest-building. Overall the crosses had lower weight-specific food consumption, and increased nest-building, body temperature, and brown adipose tissue than the purebreds. In general, heterosis in crosses between lines of different size, especially those involving large lines, tended to exceed that in crosses between lines of the same size.

1. Introduction

Historically there have been questions about the extent to which differences in body size, such as sometimes occur in north-south clines within and between closely related species, are related to temperature adaptation. More specifically, is large size associated with increased thermoregulatory advantage? Conversely, it can be asked whether differences in thermoregulation contribute to differences in growth and body size. In a previous paper, we showed that direct selection for body weight results in changes in several aspects of thermoregulation (Lynch & Roberts, 1984). Large mice have lower food consumption per gramme body weight than small mice, and this difference is closely paralleled by differences in the lipid-free weight of brown adipose tissue which probably reflects differences in weight-specific heat production. Large mice also build larger and 'better' nests, while both large and small mice maintain the same body temperature. These correlated responses in thermoregulatory traits to selection for body weight must be due to the average effects of pleiotropic genes (see Falconer, 1981, for a discussion of correlated response to selection).

The primary objective of the present study was to examine the extent to which heterosis contributes to the association between body weight and thermoregulation. Lines selected in the same direction may differ genetically among themselves because of genetic drift. By definition, however, these are not the genes affecting thermoregulation through their effect on body weight. Lines selected in opposite directions will also differ in thermoregulation because of drift, but in addition they will differ further because of those genes affecting thermoregulation pleiotropically with body weight. This experiment employed offspring from two types of crosses: between lines selected in the same direction, and between lines selected in opposite directions (including crosses between selected and control lines).

2. Materials and Methods

The experimental population was derived from the six replicates (A–F) of large, control and small mice of Falconer's Q stocks which had been selected for body weight at six weeks of age (Falconer, 1973). Selection had been suspended after 23 generations, and we employed mice from generations 62–65, at which time the coefficient of inbreeding was approximately 0·60. Largely for convenience of husbandry, crosses were made in three blocks, A × B, C × D, and E × F, so that, e.g. A lines were mated within line and with the large, control and small B lines, and reciprocal crosses were performed. Similarly, C was crossed with D, and E

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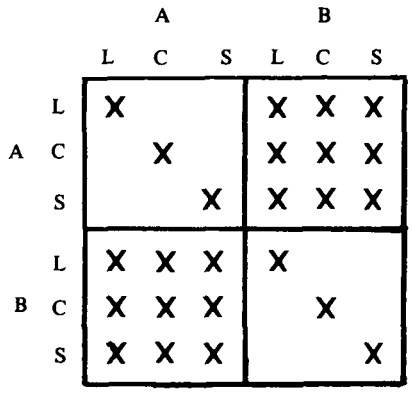

Fig. 1. One replicate of the experimental design, A and B represent replicate selection lines; L, C and S represent large, control and small lines, respectively; X indicates the crossing programme.

with F. This resulted in a replicated complete diallel between sizes, with a partial diallel between lines (see Fig. 1). The detailed crossing design is given by Bhuvanakumar *et al.* (1985).

The temperature in the mouse house averaged 22 °C, animals were exposed to a seasonally fluctuating photoperiod, and data collection spanned all seasons.

At 6 weeks of age, animals were weighed, individually housed, and nest-building and food consumption were measured for 3 days (total weight of cotton wool and food used, respectively). At 7 weeks of age, the mice were killed by cervical dislocation, core body temperature measured immediately, and the interscapular brown adipose tissue pad removed, the lipid portion extracted, and the lipid-free dry weight recorded. Methods were identical to those described more fully by Lynch & Roberts (1984). Whenever possible, two mice of each sex were sampled from each litter. A total of 1063 purebred and 1081 crossbred mice were tested. (These included the 546 crossbred and 244 purebred mice in Table 7 of Bhuvanakumar *et al.*, 1985).

Data were subjected to least-squares analyses of variance (LSML76, Harvey, 1977). Generations were treated as separate phases, so seasonal effects were contained within phase. Main effects in the analysis consisted of phase, sex, blocks, sire size, dam size, and heterosis (purebred *vs.* crossbred). Sums of squares for the complete analysis of variance were computed from several runs. Because each block comprised pure- and crossbreds from different replicate lines, block effects included random effects of genetic drift, so the appropriate main effects were tested against their interaction with blocks. In order to test for difference in within- versus between-size heterosis, additional analyses were performed on the crossbred data alone.

3. Results and Discussion

Diallel tables of least-squares means and sample sizes are given in Table 1. The striking effect of size in the crosses on all traits except body temperature, seen in row and column averages, is consistent with the differences observed in the purebreds, where larger mice build larger nests, and have less brown adipose tissue and lower food consumption per gramme body weight than smaller mice (Lynch & Roberts, 1984).

Reciprocal crosses differed for food consumption and brown adipose tissue weight, probably indicating maternal effects (Table 2). Because the latter traits are expressed in weight-specific units, maternal effects are to be expected (Bhuvanakumar *et al.*, 1985).

The heterosis exhibited in the different crosses and various averages are given in Table 2. The heterosis is also illustrated in Fig. 2, which shows the means of the crosses and the parental lines for each trait plotted against the 6-week weight of the crossbreds, so that the extent of the heterosis can be compared to the variation among the parental lines.

The difference between the crossbred and purebred means as a percentage of the purebred mean is a measure of heterosis; the values being 29·2% heterosis for total nesting score and only 0·4% for body temperature. Both traits exhibit crossbred means mostly above the range of the parental lines, and the small percentage heterosis for body temperature reflects its tight physiological regulation and consequent low coefficient of variation. Food consumption and weight of brown adipose tissue per gramme body weight gave heterosis values of −2·0% and 2·5%, respectively, the heterosis of one trait being almost the mirror image of that for the other (Fig. 2). For conparison, heterosis for 6-week body weight was 2·4% (Bhuvanakumar *et al.*, 1985). Although the differences were not significant, crosses involving large lines consistently exhibited more absolute heterosis than those involving small lines, and this was reflected in percent differences for all traits except nesting. The average heterosis among lines of different sizes also tended to exceed that for crosses within size.

Mean squares from the analyses of variance are given in Table 3. Heterosis was formally significant only for total nesting score, but the test of heterosis against the heterosis × block interaction with only 2 degrees of freedom had little power. Comparison of the mean squares for sire size (representing the effects of selection) or dam size (selection and maternal effects) or blocks (genetic drift) with that for heterosis, however, shows that body temperature also exhibited considerable heterosis compared to the effects of selection.

These data agree surprisingly well with results of a previous study of thermoregulatory traits in a very different population of mice. Analysis of a full diallel cross among four inbred strains revealed highly significant directional dominance, with crosses mostly outside the range of parental strains for nesting scores and body temperatures, while there was no significant dominance for food consumption, and 'ambidirectional dominance' (extent and direction depending on

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Table 1. *Least-squares means and sample sizes of thermoregulatory traits in crosses between lines of mice selected for 6-week body weight (B.W.). (L = large, C = control, S = small.)*

		Number of Animals				
		Crossbred				
Sire	Dam...	L	C	S	Total	Purebred
L		133	146	104	383	377
C		77	158	74	309	356
S		101	126	162	389	330
Total		311	430	340	1081	1063
		Least-squares Means*				
		Crossbred				
	Dam...	L	C	S	Mean	Purebred
Sire		Total nesting score (g)				
L		12·58	9·32	8·30	10·07	9·80
C		11·06	8·28	7·73	9·02	6·15
S		9·23	6·59	5·41	7·08	4·31
Mean		10·96	8·06	7·15	8·72	6·75
		Body temperature (°C)				
L		36·948	36·972	36·754	36·891	36·745
C		37·105	36·730	36·978	36·938	36·634
S		36·689	36·705	36·759	36·718	36·678
Mean		36·914	36·802	36·830	36·849	36·686
		Food consumption/B.W. (g/g)				
L		0·565	0·624	0·623	0·604	0·582
C		0·601	0·628	0·682	0·637	0·648
S		0·610	0·660	0·716	0·662	0·712
Mean		0·598	0·637	0·673	0·634	0·647
		Brown Adipose Tissue/B.W. (mg/g)				
L		0·446	0·488	0·520	0·485	0·430
C		0·484	0·489	0·517	0·497	0·486
S		0·492	0·501	0·546	0·513	0·543
Mean		0·474	0·493	0·528	0·498	0·486

* Means are combined across phase, sex and blocks.

the specific cross) for weight of brown adipose tissue (Lynch & Sulzbach, 1984). In an evolutionary context, extensive directional dominance is thought to reflect a history of past directional selection, with the direction of dominance indicating the direction of increasing fitness (Robertson, 1955). Presumably, then, populations of mice from which these lines were derived were adapted toward building large nests and maintaining high body temperatures. In the case of body temperature, this adaptation may have reached a selective limit, as several studies reported no significant heritability for this trait (Lacy & Lynch, 1979; Connolly & Lynch, 1983; Lynch & Sulzbach, 1984).

Among the within-size crosses, the L × L cross exhibited the largest amount of heterosis for body temperature and brown adipose tissue, and about the same amount as the C × C cross for food consumption (where the S × S cross was the least heterotic of any cross). The greater amount of heterosis among crosses involving L as compared to S lines was also observed by Bhuvanakumar *et al.* (1985) for body weight, thus, our data support their hypothesis that the frequency of dominant alleles differed more among the large lines than among the small lines.

The pattern of heterosis for traits associated with thermoregulation resulted in apparent increases in metabolic efficiency associated with increased heterozygosity. Relative to midparent values, crosses showed decreased weight-specific food consumption. Thus, while the crosses were somewhat larger, on

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Table 2. *Amounts of heterosis, defined as the deviation of the cross mean from the midparent purebred mean averaged over reciprocal crosses, together with mean amounts of heterosis for crosses between and within size of lines and overall. (L = large, C = control, S = small, B.W. = body weight.)*

Dam...	L	C	S	Mean	%
Sire	Total nesting score (g)				
L	2·78	2·22	1·71	2·24	27·0
C	—	2·13	1·93	2·09	32·4
S	—	—	1·10	1·58	28·5
Between size of line	—	—	—	1·95	—
Within size of line	—	—	—	2·00	—
Overall (±S.E.)†	—	—	—	1·97	(0·38)
Reciprocal difference	—	—	—	0·51	—
	Body temperature (°C)				
L	0·203	0·349	0·010	0·187	0·5
C	—	0·096	0·186	0·210	0·6
S	—	—	0·081	0·092	0·3
Between size of line	—	—	—	0·182	—
Within size of line	—	—	—	0·127	—
Overall (±S.E.)†	—	—	—	0·163	(0·044)
Reciprocal difference	—	—	—	−0·068	—
	Food consumption/B.W. (g/g)				
L	−0·017	−0·003	−0·031	−0·017	−2·8
C	—	−0·020	−0·009	−0·011	−1·7
S	—	—	0·004	−0·012	−1·8
Between size of line	—	—	—	−0·014	—
Within size of line	—	—	—	−0·011	—
Overall (±S.E.)†	—	—	—	−0·013	(0·012)
Reciprocal difference	—	—	—	−0·019	—
	Brown adipose tissue/B.W. (mg/g)				
L	0·016	0·028	0·020	0·021	4·6
C	—	0·003	−0·005	0·009	1·9
S	—	—	0·003	0·006	1·2
Between size of line	—	—	—	0·014	
Within size of line	—	—	—	0·007	
Overall (±S.E.)†	—	—	—	0·012	(0·007)
Reciprocal difference	—	—	—	−0·026	

* Mean reciprocal differences are listed with the cross having the largest mother given positive value.
† Approximate S.E. calculated from residual variance.

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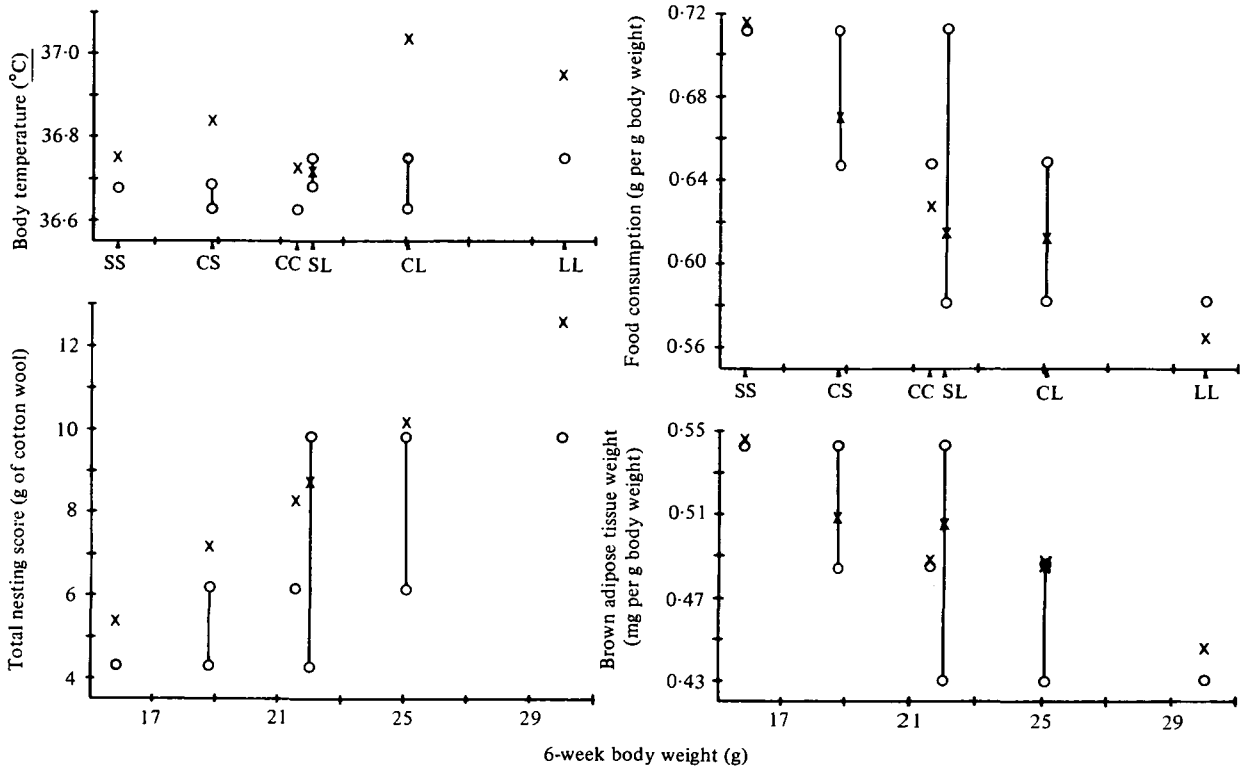

Fig. 2. Relationship between purebred and crossbred performances for the four measured variables. Means of purebreds (○) and of crossbreds (×) are plotted against the crossbred means for body weight, and all means are averaged over reciprocals and replicates. L, C and S represent large, control and small lines, respectively.

Table 3. *Mean squares from the least-squares analysis of variance for thermoregulatory traits in crosses between lines of mice selected for 6-week body weight*

Source	D.F.	Total nesting score	Body temperature	Food Cons. per g B.W.	Brown adipose wt. per g B.W.	Test vs.
Phase	3	1224·7**	27·053**	0·2226**	0·5926**	Litters
Sex	1	505·6	52·749**	2·3449**	1·6550**	Sex × B1
Blocks (B1)	2	868·2**	2·441**	0·0560	0·1795**	Litters
Sire Size (SS)	2	573·9*	1·101	0·2649	0·1805	SS × B1
Dam Size (DS)	2	1209·8	0·897	0·6159*	0·2700	DS × B1
Heterosis (Het)	1	1445·4*	9·238	0·0247	0·0584	Het × 81
Sex × B1	2	61·4*	0·301	0·0086	0·0016	Remainder
SS × B1	4	56·5	0·223	0·0476	0·0407**	Litters
DS × B1	4	257·5**	2·286*	0·0450	0·0930**	Litters
Het × B1	2	68·4*	3·226**	0·2306**	0·1425**	Litters
Residual Inter.	163	47·7**	1·163**	0·0556**	0·0261**	Remainder
Litters	380	24·6**	0·759**	0·0273**	0·0119**	Remainder
Remainder	1573	13·9	0·450	0·0091	0·0036	

* $P < 0·05$, ** $P < 0·01$.

average they consumed no more food per whole animal than the purebreds, while maintaining higher body temperatures. A nearly identical pattern of food consumption was exhibited by a smaller sample of mice measured over the entire week (Bhuvanakumar *et al.*, 1985). Weight of brown adipose tissue was slightly higher in the crosses, with the pattern of increase similar to the pattern of decreases in food consumption (Fig. 2). The association of more brown fat with lower food consumption based on heterosis is the reverse of the association based on average effects of pleiotropic genes (Lynch & Roberts, 1984; Sulzbach & Lynch, 1984). Evidence accumulating for multiple control over functional components of brown adipose tissue (e.g. Heldmaier & Buchberger, 1985), may eventually contribute to an understanding of these differences.

This research was supported by NATO grant no. 1609, the ARC Unit of the Institute of Animal Genetics, Edinburgh, and U.S. Public Health Service NIH grant no. GM21993. C.B.L. was the recipient of U.S. Public Health Service NIH Research Career Development Award no. ES00042. We

wish to thank: C. K. Bhuvanakumar for providing many of the experimental animals, Henrik Kacser for providing facilities for drying and weighing brown fat, and Peg Batchelder for collecting data on one generation. We are grateful to the staff of the Mouse House, and especially Ann Walker, for management and care of experimental animals.

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