AN INVESTIGATION OF MOUSE LINES SELECTED ON BODY WEIGHT FOR THE PRESENCE OF MAJOR GENES.

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SUMMARY

Lines of mice divergently selected for 10 week body weight were investigated for the presence of segregating alleles with large effect on weight. It appears that the X chromosome may act as a "major gene" but whether due to single or multiple allelic differences has not yet been determined. The possible role of sex-linked genes in the response to selection is considered.

INTRODUCTION

Lines of mice have been divergently selected for 20 generations on the basis of estimated lean mass in males at age 10 weeks (Hill and Bishop, 1986), and for a further 10 generations on 10 week body weight in both sexes. These lines were chosen to assess the possible contribution of major genes to their response for two reasons. Firstly, a group of genes (oncogenes) are known to have dramatic effects on cell growth and may have large effects on body weight. Secondly, major mutations affecting body weight in mice have occurred spontaneously (Bradford and Famula, 1984) and by deliberate manipulation (Palmiter *et al*, 1982).

MATERIAL AND METHODS

The progeny of a reciprocal cross between the High and Low lines were backcrossed to both parental lines. Each of the 16 families from the parental lines were, as nearly as possible, equally represented in the F1. Care was taken to ensure equal representation of each reciprocal half of the F1 in each backcross and equal representation of each family within this restriction. All individuals were weighed at 6 and 10 weeks of age. The number of families in each group (a to h; Table 1) of the backcross was reduced to five, from each of which eight individuals were kept; where possible this consisted of four from each sex. In group (b) only four families survived so six families were kept from group (d).

RESULTS

Table 1 shows 6 and 10 week body weights of each sex in the parental, F1 and backcross generations. The female is represented first in each cross, e.g "HxL" is a High female crossed to a Low male, and the subgroups of the backcrosses are identified by letters (a) to (h). The "source of F1" in the backcrosses shows from which reciprocal half of the F1 cross the F1 parent originated; this determines the sex-chromosome genotypes of the subgroups. For example, in the High backcross "HxF1" each individual will inherit a High maternal X chromosome but those from group (a), where the sire originated from the HxL reciprocal half of the F1 cross, will inherit a paternal High X or Low Y chromosome and those from group (b), where the sire originated from the LxH reciprocal half of the F1 cross, will inherit a paternal Low X or High Y chromosome.

Table 1. Means and standard deviations (g) of body weights at six and ten weeks of age in parental, F1 and backcrosses. In the crossing nomenclature H=High, L=Low and the female parent is represented first.

Parental and F1 cross:	parental HxH	F1 cross HxL	F1 cross LxH	parental LxL
female six week weight	31.9±2.73	23.7±3.67	24.6±2.29	16.5±1.77
male six week weight	35.9±3.35	30.4±3.36	25.6±2.38	19.7±2.71
female ten week weight	40.3±3.25	28.6±3.12	29.9±3.22	18.7±1.98
Male ten week weight	46.9±2.87	36.6±3.85	31.1±2.54	22.8±2.83

High backcross:	HxF1		F1xH	
Source of F1:	HxL	LxH	HxL	LxH
	(group a)	(group b)	(group c)	(group d)
Female six week weight	28.8±2.45	26.6±1.52	26.3±2.00	26.2±2.06
Male six week weight	34.1±3.37	33.4±2.46	29.7±4.23	30.1±2.40
Female ten week weight	36.0±3.45	33.3±1.76	33.2±3.67	33.2±2.83
Male ten week weight	42.6±2.71	42.0±2.93	37.3±4.50	39.8±3.43

Low backcross:	LxF1		F1xL	
Source of F1:	HxL	LxH	HxL	LxH
	(group e)	(group f)	(group g)	(group h)
Female six week weight	20.7±2.38	19.7±1.48	19.3±2.72	19.9±1.64
Male six week weight	21.7±2.70	22.7±2.62	24.8±2.79	24.4±2.18
Female ten week weight	24.6±2.99	23.1±1.97	23.0±3.07	23.1±2.45
Male ten week weight	26.8±2.68	27.6±2.88	30.4±2.34	29.0±2.82

No. of animals used:

	Parental		F1		Backcross groups							
	HxH	LxL	HxL	LxH	a	b	c	d	e	f	g	h
Females	58	41	22	30	17	15	20	25	20	21	20	20
Males	49	38	19	31	19	14	20	23	20	18	20	20

The data can be studied in detail to test predictions based on the possible modes of inheritance i.e. polygenic, autosomal major gene, sex-linked major gene, Y-chromosome effects, and maternal (cytoplasmic) inheritance. For reasons which will become obvious, the sex-linked major gene hypothesis will be considered first. There were sex differences in body weight in the F1 as the weight of males was biased towards that of the female parent. If this was a maternal effect it would be expected to affect both sexes equally but the weight of females is midway between that of the parental lines in both halves of the reciprocal cross. A major difference in genotype between the sexes is that males receive only a maternal X chromosome, so initial inspection of the data suggested that this

chromosome may have a significant effect on body weight. This hypothesis generates four predictions in the backcross based on the fact that males receive only one X chromosome which is transmitted maternally. In the High backcross males from "HxF1" (groups a+b) all inherit a High X chromosome so should be heavier than those from the reciprocal "F1xH" (groups c+d) which may inherit either type of X chromosome. Their phenotypic standard deviation (SD) should be lower since only one type of X chromosome is present. Similarly, in the Low backcross, males from "LxF1" (groups e+f) all inherit the Low X chromosome so should be lighter than their reciprocal counterparts (groups g+h); their SD should also be lower. In the high backcross the females from "HxF1" should be heavier in matings where the sire carries the High X chromosome (group a) since they will be homozygous "High X" rather than heterozygous (group b). Similarly, in the equivalent Low backcross "LxF1"; females in group (e) should be heavier since they are heterozygous for X chromosomes rather than homozygous "Low X" in group (f). The data appear consistent with these predictions. The data suggest the X chromosome has a semi-dominant effect on body weight in the order of 5g when hemizygous or homozygous and 2.5g when heterozygous at age 10 weeks. This represents 22% and 11% of the divergence in body weight. The effect was also apparent at age 6 weeks where the equivalent figures are 4g (hemi- or homozygous) and 2g (heterozygous) which represent 25% and 13% respectively of the divergence in body weight.

Attempts to detect the presence of autosomal major genes must allow for the putative presence of sex-linked effects. Body weights in females from the F1 are midway between the parental values which is consistent both with a polygenic or autosomal semi-dominant gene. The presence of a major gene segregating in the backcross can be detected by increases in the coefficient of variation (c.v., standard deviation/mean) relative to the F1. The presence of possible sex linked effects restricts the comparison to groups (a), (b), (e) and (f), within which each individual has the same X chromosome genotype. No increase in c.v. was evident so it is doubtful if an autosomal gene with a large phenotypic effect could have been present.

The data can also be investigated to detect major effects on body weight associated with the Y chromosome. In the F1 cross the effects of the X and Y chromosomes cannot be separated. Males in groups (a) and (b) of the High backcross should have similar genotypes except that the Y chromosome was derived from the Low and High lines respectively. Similarly in the Low backcross, males in groups (e) and (f) will contain Y chromosomes derived from the Low and High lines respectively. The lack of discrepancy in body weight between groups (a) and (b) and between groups (e) and (f) suggest that no significant effects were associated with the Y chromosome.

There appears to be no maternal *in utero* effects or indications of cytoplasmic or mitochondrial inheritance. Consideration of these effects yield three predictions which are not fulfilled. The females in the F1 contain an X chromosome inherited from each parental line. However the females from "HxL" half of the cross will also inherit the high cytotype and should be larger. In the High backcross there should be differences in "F1xH" depending on whether the F1 female inherited the High (group c) or Low (group d) cytotype. Similarly in the Low backcross there should be differences in "F1xL" depending on whether a High maternal cytotype (group g) or Low maternal cytotype (group h) was inherited.

DISCUSSION

On the basis of these data it is impossible to distinguish whether there is a single sex-linked major gene or whether the X chromosome contains a number of segregating alleles at loci affecting body weight. The X chromosome is associated with approximately 25% of the High/Low divergence in body weight at 6 and 10 weeks of age but accounts for only 5% to 6% of the haploid DNA in mice (Ohno, 1967). Sex-linked genes are dominant in the heterogametic sex (males in mammals, females in birds) so selection on recessive or semi-dominant alleles on the sex chromosomes will be more effective than on autosomal alleles, and their contribution to response will be proportionally larger, particularly since selection in these lines was practised only on males during the first 20 generations. A polygenic response in body weight may therefore explain the possible "major gene" effect of the sex chromosome. The magnitude of such biases will depend on their dominance relationships for the selected trait, the relative genetic variance at autosomal and sex-linked loci (Charlesworth *et al.* 1987), their relative mutation rates (Miyata *et al.* 1987), and the contribution of new mutations to the response (Hill, 1982). Such biases will affect analyses based on calculating the resemblance between relatives (James, 1973; Grossman and Eisen, 1989). Biases in the contribution of sex-chromosomes to heterosis were noted by Stonaker (1963), although subsequent work has been inconclusive (White *et al.* 1970).

The proportion of DNA in the sex chromosome and its gene content is similar in all mammalian species (Ohno, 1967; Lalley and M^{c}Kusick, 1985) so a bias noted in mice may occur in other species. Assuming that alleles influencing growth traits are spread at random throughout the genome, that the sex chromosome contains 6% of the genome but contributes 25% to the response, realized heritability is predicted to be 19% higher in the heterogametic sex; a result with significant commercial implications. The possibility of a disproportionate contribution of sex-linked alleles in the response to selection may therefore reward further investigation. Alternatively, the presence of a sex-linked major gene allows biochemical studies with the possible identification of a locus suitable for direct manipulation. Future work will attempt to confirm the apparent "major gene" effect of the X chromosome, and to identify its genetic basis.

Acknowledgements : This work was supported by the Agricultural and Food Research Council.

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