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Y-chromosomal and other factors in the development of testis size in mice

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Summary

Testis size was investigated in two inbred strains of mice, BALB/c/Ola and CBA/Gr, at different ages. BALB/c mice were found to have the larger testes from day 14 of embryonic development onwards. Body weights of the two strains differed to a lesser extent. The differences in testis weights were analysed post-natally at 2, 4, 6 and 8 weeks in the two strains, F_1s, F_2s and backcrosses. Testis size was found to be affected by the origin of the *Y* chromosome, the *X* chromosome, the autosomes (and/or pseudoautosomal regions) and by maternal factors. At 8 weeks of age, the *Y*-chromosomal effect was at its strongest, and the *X*-chromosomal effect was at its weakest, while the maternal effect had vanished. It is postulated that non-*Y*-chromosomal factors which modify testis size may affect the gonads in both sexes. The possibility is discussed that loci affecting gonad size may be identical with testis-determining factors.

1. Introduction

Among genetically determined quantitative variables, testis size occupies a place of special importance because of its direct connexion with reproduction and fertility. Mice of the C57BL/10J strain have small testes, associated with androgen deficiency and reduced levels of spermatogenesis (Shire & Bartke, 1972). The genetic effects, moreover, are not confined to the male, since testis size in mice has been found to be correlated with ovulation rates (Land, 1973; Islam, Hill & Land, 1976), while in humans a correlation between testis size and dizygotic twinning has been suggested (Short, 1984; Diamond, 1986).

In mice, the major causes affecting testis size appear to be genetic (Hunt, 1986). Hayward & Shire (1974) have presented evidence of a *Y*-chromosomal effect on adult testis weight in two inbred strains of mice, and Stewart (1983) speaks of 'allelic variation' of the *Y* chromosome with regard to its effect on testis weight. However, Islam *et al.* (1976) thought it unlikely that a large proportion of the genetic variation in testis weight is controlled by the *Y* chromosome, since selection for testis size was found to be accompanied by a correlated response in ovulation rate; and Herrick & Wolfe (1977) found that while the *Y* chromosome did affect testis weight in their strains of mice, the major component of genetic variation appeared to be autosomal.

We now wish to report data on testis size in two inbred strains of mice, BALB/c/Ola and CBA/Gr (henceforth referred to as BALB/c and CBA resp.). The two strains also differed in body weight, though to a lesser extent. The relationship between testis weight and body weight is complex, and differs at different ages. Whereas body weight has a rather complicated biological basis, being the sum of the weights of the skeleton, muscles, organs and fat, testes are relatively homogeneous organs, consisting of spermatogenic and two types of somatic cells. Androgenic hormones produced by the testis are known to affect components of body weight. Testis weight is positively correlated with sperm count (Krzanowska, 1971), and the absolute sperm count is likely to be biologically more meaningful than a quantity related to notional body weight. In this paper we present both uncorrected and corrected testis weights.

2. Material and Methods

The BALB/c and CBA strains were chosen for investigation after preliminary experiments demonstrated a substantial difference in adult testis size, as well as good breeding performance. Male mice aged 2, 4, 6 and 8 weeks were killed by etherization and weighed on a Mettler scale. Gonads were dissected out and weighed separately on an Oertling scale to the nearest 0·01 mg. Epididymal sperm counts were made by a modification of the method of Searle & Beechey (1974).

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Table 1. *Testis weights and body weights in BALB/c and CBA mice aged 2–8 weeks*

Age (weeks)	Mean testis weight[a] (mg ± S.E.)[a]			Mean body weight (g ± S.E.)		
	BALB/c	CBA	Difference (%)[b]	BALB/c	CBA	Difference (%)[c]
2	11·6 ± 0·2	7·4 ± 0·2	36	8·4 ± 0·3	5·6 ± 0·2	33
4	55·7 ± 2·8	29·5 ± 1·7	47	17·4 ± 0·3	13·2 ± 0·5	24
6	90·2 ± 1·0	51·0 ± 3·2	43	21·2 ± 0·8	18·1 ± 0·7	15
8	100·0 ± 1·6	62·8 ± 2·3	37	23·8 ± 0·4	21·8 ± 0·3	8

[a] Mean of mean of both testes. [b]Calculated as [(BALB/c − CBA)/BALB/c] × 100. Numbers of animals in each group are shown in Tables 4–7.
[b] Differences significant at 0·001 level at all ages.
[c] Differences at 2 and 4 weeks significant at 0·001 level, at 6 weeks at 0·05 level and 8 weeks at 0·01 level.

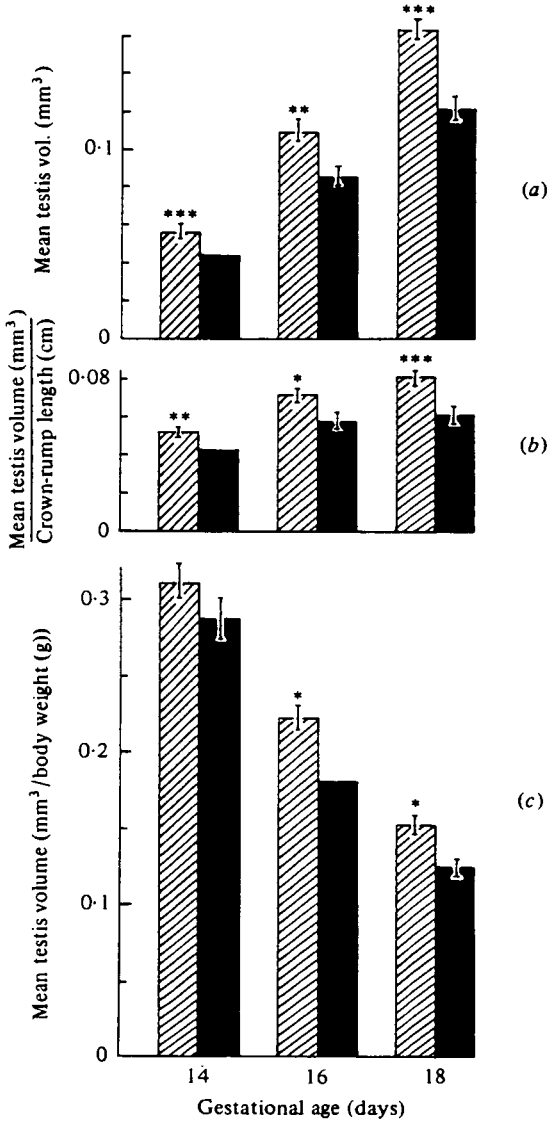

Fig. 1. Testicular volumes in foetuses of BALB/c (cross-hatched) and CBA (solid block). (*a*) Absolute volumes; (*b*) testicular volumes in relation to crown–rump length; (*c*) testicular volumes in relation to body weight. * $P < 0·01$; ** $P < 0·002$; *** $P < 0·001$.

For the assessment of testicular volumes before birth, the mother was killed by etherization on the 14th, 16th or 18th day of pregnancy (day of plug being counted as day 0) and embryos were dissected out. Crown–rump lengths were measured prior to fixation in Bouin's solution. Fixed gonads were dissected out from embryos aged 16 and 18 days, and left *in situ* in 14-day-old embryos. Serial sections were cut at 7 μm and stained with haematoxylin and eosin. Section areas were measured, and volumes estimated, using the method of Mittwoch, Mahadevaiah & Setterfield (1984). Statistical analysis was carried out by Student's *t* test, and by maximum likelihood analysis (Table 3).

3. Results

(i) *Testis weights and body weights*

Mean testis weights and body weights of BALB/c and CBA mice aged 2–8 weeks are shown in Table 1. It is evident that the weights of BALB/c testes exceed those of the CBA strains at all age groups examined, the differences varying between 36 and 47%. Likewise, BALB/c mice exceed CBA mice in body weight, but to a lesser extent, the differences ranging from 33% at 2 weeks to 8% at 8 weeks of age. At 4–8 weeks of age the difference in testis weights between the two strains is markedly greater than that for body weight, whereas at 2 weeks of age the two differences are not significantly different. This, however, cannot be taken to mean that the difference in testis weight (as distinct from body weight) only begins to develop at this time, since volumetric measurements of foetal testes, illustrated in Fig. 1, have shown a significant difference in testis size between strains at 14 days gestational age, and in testis volume relative to body weight at 16 days.

It can be seen from Fig. 2 that in adults the difference in testis weight is reflected in the difference in sperm numbers and in caput weights, and that both parameters are more than twice the difference in body weight.

(ii) *Analysis of postnatal testis weights in BALB/c, CBA, F_1s, F_2s and backcrosses*

Table 2 summarizes the strains and crosses used to analyse the genetic components affecting testis weight

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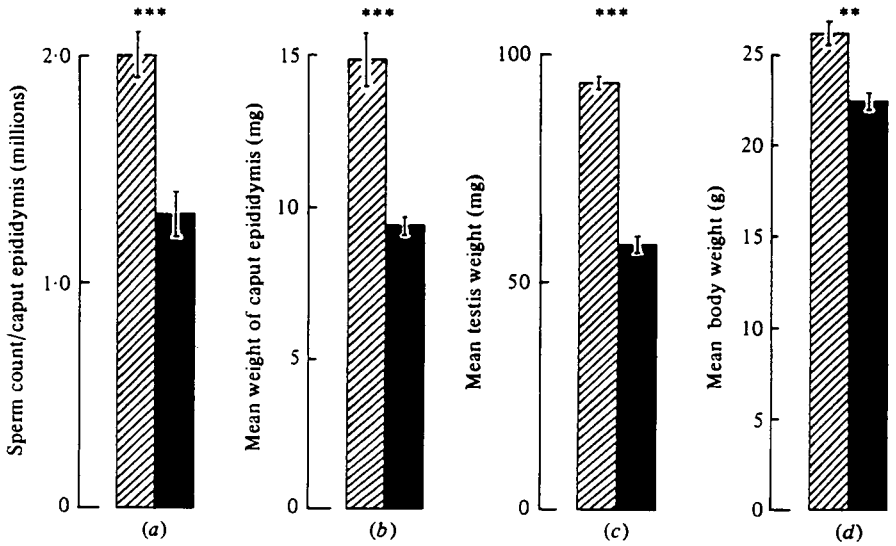

Fig. 2. Sperm counts, epididymal weights, testis weights and body weights in BALB/c (cross-hatched) and CBA (solid block) aged 8 weeks.

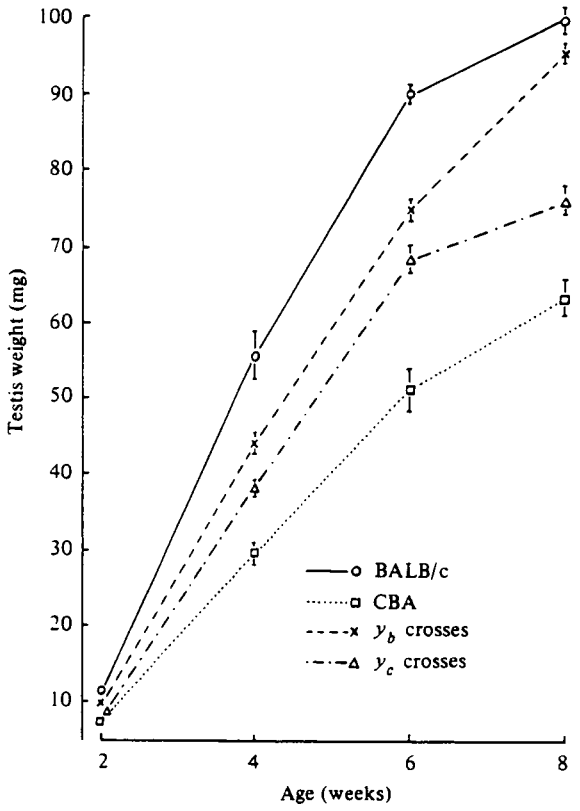

Fig. 3. Testis weights of strains and hybrids of ages 2–8 weeks. Points labelled y_b are unweighted means of all F_1, F_2 and backcross generations containing BALB/c Y chromosome, Y_c similarly for CBA.

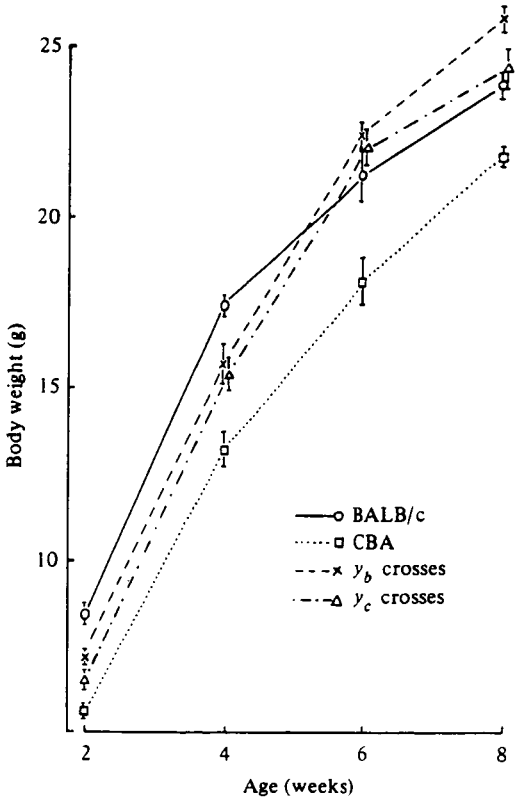

Fig. 4. Body weights of strains and hybrids of ages 2–8 weeks. y_b and y_c as for Fig. 3.

at 2, 4, 6 and 8 weeks, and also lists the genetic sources accounting for the differences between paired comparisons. Data on testis weights are shown in Tables 4–7 and are illustrated in Fig. 3, while body weights are shown in Fig. 4. It will be seen that the testis weights of hybrids lie between those of parental strains, whereas body weights exhibit heterosis from 6 weeks of age onwards.

It is evident from Table 2 that the two groups, i.e. with Y chromosomes derived from BALB/c (Y_B) and from CBA (Y_C) respectively, are balanced as regards the derivation of their X chromosome and their maternal contribution, but that there is an autosomal imbalance, the Y_B group containing 25% too many BALB/c autosomes and the Y_C group containing 25% too many CBA autosomes. This is corrected by the maximum likelihood design (Table 3), in which values attributable to CBA components are subtracted from those attributable to BALB/c values.

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Table 2. *Summary of available strains and crosses*

Generations	Parents: *Y* from BALB/c ♂ × ♀	*Y* from CBA ♂ × ♀	Factors in reciprocal difference	Direction of difference (all Y_B–Y_C)
(1,9) P	B B	C C	*Y*, *X*, A, M	X_B–X_C, A_B–A_C, M_B–M_C
(2,10) F_1	B C	C B	*Y*, *X*, M	X_C–X_B, M_C–M_B
(3,11) F_2	(B × C) (B × C)	(C × B) (C × B)	*Y* M	M_C–M_B
(4,12) F_2	(B × C) (C × B)	(C × B) (B × C)	*Y* M	M_B–M_C
(5,13) Bcr	B (B × C)	(C × B) B	*Y*, $\frac{1}{2}X$, M	$\frac{1}{2}X_C$–$\frac{1}{2}X_B$, M_C–M_B
(6,14) Bcr	(B × C) B	(C × B) C	*Y*, *X*, $\frac{1}{2}$A, M	X_B–X_C $\frac{1}{2}A_B$–$\frac{1}{2}A_C$ M_B–M_C
(7,15) Bcr	B (C × B)	C (C × B)	*Y*, $\frac{1}{2}$A,	$\frac{1}{2}A_B$–$\frac{1}{2}A_C$
(8,16) Bcr	(B × C) C	C (B × C)	*Y*, $\frac{1}{2}X$,	$\frac{1}{2}X_C$–$\frac{1}{2}X_B$
Total	$8Y_B$; $4X_B$; $4X_C$; $2M_B$; $2M_C$; $2M_{B\times C}$; $2M_{C\times B}$; $10A_B$; $6A_C$;	$8Y_C$; $4X_B$; $4X_C$; $2M_B$; $2M_C$; $2M_{B\times C}$; $2M_{C\times B}$; $6A_B$; $10A_C$		

B = BALB/c; C = CBA; *Y* = *Y* chromosomes; *X* = *X* chromosome; A = autosomes (including pseudoautosomal regions); M = maternal effect; Bcr = backcross.

Table 3. *Design matrix of maximum-likelihood analysis* (*the convention is that crosses are male × female*)

d[1, A]: = $\frac{1}{2}$;	d[1, X]: = $\frac{1}{2}$;	d[1, M]: = $\frac{1}{2}$;	d[1, *Y*]: = $\frac{1}{2}$;	{P B}
d[2, A]: = 0;	d[2, X]: = $-\frac{1}{2}$;	d[2, M]: = $-\frac{1}{2}$;	d[2, *Y*]: = $\frac{1}{2}$;	{F_1 B × C}
d[3, A]: = 0;	d[3, X]: = 0;	d[3, M]: = $-\frac{1}{2}$;	d[3, *Y*]: = $\frac{1}{2}$;	{F_2 (B × C) × (C × B)}
d[4, A]: = 0;	d[4, X]: = 0;	d[4, M]: = $\frac{1}{2}$;	d[4, *Y*]: = $\frac{1}{2}$;	{F_2 (B × C) × (C × B)}
d[5, A]: = $\frac{1}{4}$;	d[5, X]: = 0;	d[5, M]: = $-\frac{1}{2}$;	d[5, *Y*]: = $\frac{1}{2}$;	{Bcr B × (B × C)}
d[6, A]: = $\frac{1}{4}$;	d[6, X]: = $\frac{1}{2}$;	d[6, M]: = $\frac{1}{2}$;	d[6, *Y*]: = $\frac{1}{2}$;	{Bcr (B × C) × B}
d[7, A]: = $\frac{1}{4}$;	d[7, X]: = 0;	d[7, M]: = $\frac{1}{2}$;	d[7, *Y*]: = $\frac{1}{2}$;	{Bcr B × (C × B)}
d[8, A]: = $-\frac{1}{4}$;	d[8, X]: = $-\frac{1}{2}$;	d[8, M]: = $-\frac{1}{2}$;	d[8, *Y*]: = $\frac{1}{2}$;	{Bcr (B × C) × C}
d[9, A]: = $-\frac{1}{2}$;	d[9, X]: = $-\frac{1}{2}$;	d[9, M]: = $-\frac{1}{2}$;	d[9, *Y*]: = $-\frac{1}{2}$;	{P C}
d[10, A]: = 0;	d[10, X]: = $\frac{1}{2}$;	d[10, M]: = $\frac{1}{2}$;	d[10, *Y*]: = $-\frac{1}{2}$;	{F_1 C × B}
d[11, A]: = 0;	d[11, X]: = 0;	d[11, M]: = $\frac{1}{2}$;	d[11, *Y*]: = $-\frac{1}{2}$;	{F_2 (C × B) × (C × B)}
d[12, A]: = 0;	d[12, X]: = 0;	d[12, M]: = $-\frac{1}{2}$;	d[12, *Y*]: = $-\frac{1}{2}$;	{F_2 (C × B) × (B × C)}
d[13, A]: = $\frac{1}{4}$;	d[13, X]: = $\frac{1}{2}$;	d[13, M]: = $\frac{1}{2}$;	d[13, *Y*]: = $-\frac{1}{2}$;	{Bcr (C × B) × B}
d[14, A]: = $-\frac{1}{4}$;	d[14, X]: = $-\frac{1}{2}$;	d[14, M]: = $-\frac{1}{2}$;	d[14, *Y*]: = $-\frac{1}{2}$;	{Bcr (C × B) × C}
d[15, A]: = $-\frac{1}{4}$;	d[15, X]: = 0;	d[15, M]: = $\frac{1}{2}$;	d[15, *Y*]: = $-\frac{1}{2}$;	{Bcr C × (C × B)}
d[16, A]: = $-\frac{1}{4}$;	d[16, X]: = 0;	d[16, M]: = $-\frac{1}{2}$;	d[16, *Y*]: = $-\frac{1}{2}$;	{Bcr C × (B × C)}

P = parental; Bcr = backcross; A = autosomes; *X* = *X* chromosome; M = maternal contribution; *Y* = *Y* chromosome; B = BALB/c; C = CBA.

d[1, A] means (proportion of autosomes derived from BALB/c $-\frac{1}{2}$) in cross 1. d[1, X] similarly refers to *X* chromosomes, etc.

Table 4. *Mean testis* (± S.E.) *weights of BALB/c, CBA, F_1, F_2 and backcrosses at 2 weeks of age. (For details of crosses and abbreviations see Table 2)*

Generations	*Y* from BALB/c *N*	*Y* from BALB/c Testis wt. (mg)	*Y* from CBA *N*	*Y* from CBA Testis wt. (mg)	Testis wt. (mg)/body wt. (100 g): *Y* from BALB/c	*Y* from CBA
(1,9) P	10	11·6 ± 0·3	9	7·4 ± 0·2	139 ± 5·2	133 ± 5·3
(2,10) F_1	6	9·1 ± 0·2	5	9·0 ± 0·4	140 ± 4·9	161 ± 1·0
(3,11) F_2	6	9·2 ± 0·3	6	7·9 ± 0·6	138 ± 2·6	110 ± 4·2
(4,12) F_2	6	8·6 ± 0·5	6	11·0 ± 0·4	130 ± 6·1	149 ± 7·5
(5,13) Bcr	5	9·9 ± 0·4	6	6·5 ± 0·9	144 ± 3·4	123 ± 8·3
(6,14) Bcr	6	10·0 ± 0·3	6	7·8 ± 0·6	128 ± 4·5	110 ± 4·3
(7,15) Bcr	6	10·5 ± 0·4	6	8·5 ± 0·3	128 ± 5·7	120 ± 2·2
(8,16) Bcr	6	12·1 ± 0·7	6	9·9 ± 0·6	172 ± 9·2	143 ± 2·3

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Table 5. *Mean testis weights (±S.E.) in BALB/c, CBA, F_1, F_2 and backcrosses generations at 4 weeks of age. (For details of crosses and abbreviations see Table 2)*

	Y from BALB/c		*Y* from CBA		Testis wt. (mg)/body wt. (100 g)	
Generations	*N*	Testis wt. (mg)	*N*	Testis wt. (mg)	*Y* from BALB/c	*Y* from CBA
(1,9) P	6	55·7±2·8	6	29·5±1·7	319±12·4	223±6·1
(2,10) F_1	5	44·5±3·0	6	39·5±1·4	281±6·3	230±2·4
(3,11) F_2	6	49·2±1·4	6	37·1±3·4	287±8·5	240±15·9
(4,12) F_2	6	36·2±1·9	6	42·1±3·8	272±9·1	272±12·0
(5,13) Bcr	6	45·0±2·7	6	37·6±2·5	299±6·4	251±9·6
(6,14) Bcr	6	50·2±3·5	6	34·2±2·0	299±13·0	219±8·8
(7,15) Bcr	6	46·7±2·7	6	33·7±1·9	280±8·9	230±9·3
(8,16) Bcr	6	36·8±3·0	6	43·4±2·7	269±10·8	254±8·1

Table 6. *Mean testis weights (±S.E.) in BALB/c, CBA, F_1, F_2 and backcrosses generations at 6 weeks of age. (For details of crosses and abbreviations see Table 2)*

	Y from BALB/c		*Y* from CBA		Testis wt. (mg)/body wt. (100 g)	
Generations	*N*	Testis wt. (mg)	*N*	Testis wt. (mg)	*Y* from BALB/c	*Y* from CBA
(1,9) P	6	90·2±1·0	6	51·0±3·2	430±20·5	281±9·0
(2,10) F_1	6	78·0±3·8	6	75·7±2·4	339±5·4	326±13·4
(3,11) F_2	6	75·3±1·0	6	54·4±6·8	337±11·0	253±26·9
(4,12) F_2	6	63·1±4·5	6	76·6±7·7	283±16·3	332±39·8
(5,13) Bcr	6	78·0±1·9	6	67·7±3·1	349±4·8	300±17·8
(6,14) Bcr	6	79·2±4·1	6	60·3±1·8	361±7·0	272±5·5
(7,15) Bcr	6	80·2±4·2	6	65·6±2·9	328±5·7	284±7·6
(8,16) Bcr	6	68·5±3·1	6	75·3±2·1	316±11·2	338±6·7

Table 7. *Mean testis weights (±S.E.) in BALB/c, CBA, F_1, F_2 and backcrosses generations at 8 weeks of age. (For details of crosses and abbreviations see Table 2)*

	Y from BALB/c		*Y* from CBA		Testis wt. (mg)/body wt. (100 g)	
Generations	*N*	Testis wt. (mg)	*N*	Testis wt. (mg)	*Y* from BALB/c	*Y* from CBA
(1,9) P	6	100·0±1·6	5	62·8±2·3	419±4·3	288±8·3
(2,10) F_1	5	94·2±1·3	6	80·7±1·2	359±4·8	292±7·8
(3,11) F_2	6	93·7±2·5	6	77·7±5·6	366±12·3	324±20·7
(4,12) F_2	6	96·1±3·5	6	80·9±6·3	381±14·8	344±22·0
(5,13) Bcr	6	98·7±3·6	6	79·8±5·4	369±8·9	329±19·1
(6,14) Bcr	5	97·7±3·2	6	70·9±2·3	359±18·0	292±15·1
(7,15) Bcr	6	96·1±3·3	6	74·3±3·5	365±11·6	311±12·9
(8,16) Bcr	6	91·4±4·1	6	70·8±3·4	371±16·1	271±7·4

The results showing the relative contributions of autosomal (including pseudoautosomal), *X*-chromosomal, *Y*-chromosomal and maternal factors on testis weight are shown in Table 8. The percentage values are a measure of the relative influence of the genetic component under consideration on the difference in testis size between the two strains.

The χ^2 values probably overestimate significance levels somewhat because the model does not take account of the differences in variance between F_1, F_2 and backcrosses. In order to correct for this, $F_{1/11}$ values were calculated by dividing the residual sum of squares by the number of degrees of freedom. It is thought that the $F_{1/11}$ values are likely to underestimate significance levels and that the true significance levels lie between those obtained from χ^2 and $F_{1/11}$ values.

The data in Table 8 suggest that the autosomally inherited effect on the difference in testis weights between the two strains accounts for about 35–46% throughout, whereas *X*-chromosomal and maternal effects appear to diminish with age, the latter having vanished at 8 weeks. The maternal effect differs from the other three in direction, i.e. the maternal effect of BALB/c is to diminish testis weight.

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Table 8. *Maximum likelihood analysis estimating respective contributions of autosomal, X-chromosomal, Y-chromosomal and maternal factors on the difference in testis weights between BALB/c and CBA*

Age (weeks)	Proportional effect (%) of				Mean difference (mg) in testis weight (BALB/c − CBA)
	Autosomes	*X*-chromosome	*Y*-chromosome	Maternal effects	
2	35 $\chi^2_1 = 3$ $F_{1/11} = 0{\cdot}27$	47 $\chi^2_1 = 12^{***}$ $F_{1/11} = 1{\cdot}09$	31 $\chi^2_1 = 16^{***}$ $F_{1/11} = 1{\cdot}45$	−31 $\chi^2_1 = 15^{***}$ $F_{1/11} = 1{\cdot}36$	4·2
4	46 $\chi^2_1 = 6{\cdot}5^{*}$ $F_{1/11} = 3{\cdot}28$	39 $\chi^2_1 = 6{\cdot}5^{*}$ $F_{1/11} = 3{\cdot}28$	22 $\chi^2_1 = 8^{**}$ $F_{1/11} = 4{\cdot}06$	−26 $\chi^2_1 = 11^{***}$ $F_{1/11} = 5{\cdot}58^{*}$	26·2
6	39 $\chi^2_1 = 33^{***}$ $F_{1/11} = 1{\cdot}46$	37 $\chi^2_1 = 76^{***}$ $F_{1/11} = 3{\cdot}37$	21 $\chi^2_1 = 110^{***}$ $F_{1/11} = 1{\cdot}73$	−17 $\chi^2_1 = 39^{***}$ $F_{1/11} = 1{\cdot}73$	39·2
8	39 $\chi^2_1 = 56^{***}$ $F_{1/11} = 6{\cdot}59^{*}$	10 $\chi^2_1 = 5$ $F_{1/11} = 0{\cdot}59$	46 $\chi^2_1 = 779^{***}$ $F_{1/11} = 91{\cdot}65^{***}$	0	37·2

$^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

3. Discussion

In a comparison of testis weights between two inbred strains of mice, SF and CBA, Hayward & Shire (1974) estimated that 41 % of the difference was attributable to factors on the *Y* chromosome. Although in the present study we used BALB/c mice and a different substrain of CBA, our estimate of 46% of the difference in testis weights of 8-week-old mice being due to *Y*-chromosomal effects (Table 8) is clearly similar, which suggests that the *Y* chromosomes of BALB/c and SF have equal effects on testis size. Our study suggests that the *Y* chromosome affects testis weight also in younger mice aged 2–6 weeks, but to a lesser extent. The data further suggest that BALB/c autosomes have an effect of increasing testis weight, although results at early ages did not reach statistical significance. It is likely, of course, that experimental errors will be relatively greater the smaller the testes that are weighed. Taken at their face value, the data indicate that about 40% of the difference in testis weight between the two strains could be due to autosomal or pseudoautosomal effects. On the assumption that there is an obligatory chiasma leading to crossing over within the pairing region of the *X* and *Y* chromosomes in the mouse, the inheritance of loci distal to the chiasma will be indistinguishable from autosomal transmission (Burgoyne, 1982). The effects attributable to the non-homologous region of the *X*-chromosome seemed to diminish with age, and the same applies to maternal factors, which were detectable between 2 and 6 weeks of age, but not at 8 weeks.

The maternal effect differed from the chromosomal ones in direction, since factors associated with BALB/c mothers led to diminished testis weights. It is tempting to speculate that the maternal effects might be due to a difference in mitochondria. This idea is supported by the observed testis weights in reciprocal F_2s, aged two to six weeks: testis weights were consistently higher in the offspring of mothers who had contributed CBA cytoplasm, even though chromosomal components were constant (see Tables 4–7, generations 3 and 4). It could be relevant that Wilkie *et al.* (1983) have presented evidence that an alteration to mitochondria could affect the activity of nuclear genes involved in the biogenesis of cell-surface components.

Our finding that testis size in mice is affected by factors on the *X* and *Y* chromosomes, the autosomes (and/or pseudoautosomal regions), as well as by maternal factors may help to explain the apparently varying results obtained by previous authors. Whereas Hayward & Shire (1974) stressed the *Y*-chromosomal effect on testis size in mice, Islam *et al.* (1976) found a significant correlation between testis size and ovulation rates in females and concluded that it was unlikely that a large proportion of the genetic variation in testis weight was controlled by the *Y*-chromosome. Herrick & Wolfe (1977) also thought that the major genetic variation in testis weight was due to autosomal factors, even though their data also suggested a *Y*-chromosomal effect. It would seem that in all cases described so far the effect of the *Y* chromosome accounted for less than half of the genetic difference in testis weight, so that non-*Y*-chromosomal factors must also be involved. The latter could affect the gonads in both sexes.

So far, the discussion has been concerned with allelic variation giving rise to differences in testis size between strains, and not with the nature of the loci which exhibit this allelic variation. Clearly, the *a priori* assumption would be that these loci affect testis size.

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In Hunt's (1986) data, testis weights exceeded ovary weights by a factor of 20 in litter-mates of four strains. In such comparisons, autosomal and maternal factors can be discounted and the genetic difference becomes reduced to presence versus absence of a *Y* chromosome, and to the presence of one versus two *X* chromosomes. There can be little doubt that the major difference is connected with the presence of a *Y* chromosome. Results obtained by Mittwoch & Buehr (1973) have shown that, in mouse foetuses aged 16 days from litters segregating for *Sxr*, the difference between one and two *X* chromosomes can have had only a minor effect on gonad size, whereas the major effect was associated with the presence of a *Y* chromosome or of *Sxr*. However, while in the case of allelic variation associated with different phenotypes the relationship between cause and effect is unambiguous, the causal relationship between the presence of a *Y* chromosome and a large testis, compared with the absence of a *Y* and a small ovary, is less straightforward, since it could be argued that the large size of the testis is merely a result of its differentiation and hence unrelated to *Y*-chromosomal loci concerned with growth. On the other hand, Mittwoch (1973) proposed that accelerated gonadal growth is a *Y*-chromosomal effect and a *sine qua non* for testicular differentiation. The concordant relationship between bilateral asymmetry of gonadal growth in human foetuses and of gonadal differentiation in hermaphrodites (Mittwoch, 1986) supports this view. The process of gonadal differentiation in mammals could then be seen as resulting from a threshold dichotomy, and the *Y*-chromosomal and non-*Y*-chromosomal factors affecting gonad size could be identical with sex-determining genes. It is relevant in this connexion that data on sex reversal in mice reported by Eicher *et al.* (1982) and by Washburn & Eicher (1983) point to the involvement of two non-*Y*-chromosomal loci, at least one of which is autosomal. It will be important to test whether these loci also affect gonad size.

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