Genetic analysis of the *Y*-chromosome of the mouse: evidence for two loci affecting androgen metabolism

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Summary

Male mice from congenic lines carry *Y*-chromosomes derived from two pairs of inbred strains (CBA/FaCamSt and C57BL/FaSt; PHL/St and PHL-YH/St) on various genetic backgrounds were compared. Serum testosterone levels, and the response of target organs in castrated animals to graded doses of exogenous testerone propionate were measured. These comparisons produced evidence for two *Y*-chromosomal loci influencing androgen metabolism. One of these affects serum testosterone levels, with variant alleles on the *Y*-chromosomes derived from the PHL and PHL-YH strains. The other locus influences the response to testosterone of target organs, most significantly seminal vesicle, and variant alleles are found in the CBA and C57 strains. The effects of both loci are modulated by the genetic background. The relationship of these loci to other *Y*-chromosomal loci in the mouse is briefly discussed.

1. Introduction

Variation between inbred strains of the mouse in both blood androgen levels and the sensitivity of target organs to androgens has been well documented (for example, Bartke, 1974; Batty, 1978; Chai & Dickie, 1966; Amos & Stewart, 1980; West, Evans & Hamilton, 1980); but the possibility of a *Y*-chromosomal contribution to this variation has not been systematically investigated. There is increasing evidence that the mammalian *Y*-chromosome has actions in the male additional to that of triggering testis determination in embryonic development (reviewed by Stewart, 1983). A possible effect on testosterone levels of the *Y*-chromosomes derived from two different inbred mouse lines was reported by Selmanoff *et al.* (1977*a*,*b*). We therefore undertook a study of the role of *Y*-chromosome origin in determining serum testosterone levels, the weights of androgen target organs in intact animals, and the sensitivity of target organs to exogenous androgens. Preliminary reports of some of these results have been published (Jutley & Stewart, 1981; 1984).

2. Materials and Methods

(i) *Mice*

All the mice were bred in the University of Leeds Laboratory Animal Unit, and fed on Labsure, C.R.M. mouse diet. The mice were weaned at 3 weeks of age, sexes separated, and kept in cages 26 × 19 × 10 cm at a density of less than 10 mice per cage. They were maintained at 20 ± 2 °C on a reverse lighting schedule (12 hr light, 12 hr dark) throughout the year.

Inbred strains of mice for comparison were chosen which were already known to show *Y*-chromosomal effects on other characters, including both testis weight and the sex ratio of offspring (Hayward & Shire, 1974; Weir, 1960, 1976).

The strains of mice used were (i) CBA/FaSt, (ii) C57BL/FaSt, (iii) PHL/St, (iv) PHL-YH/St. Comparisons of the effects of the *Y*-chromosomes were made between lines derived from (i) and (ii), and from (iii) and (iv). The designations to be used for the strains and their *Y*-chromosomes are shown in Table 1. In order to compare the *Y*-chromosomes of two strains it is necessary to have congenic lines differing only in their *Y*-chromosomes. For comparison of the CBA and C57 *Y*-chromosomes, the two strains were crossed and reciprocal F1 males were backcrossed to each of the parental strains. This provided a strain congenic with CBA but carrying the *Y*-chromosome from C57, and a strain congenic with C57 but carrying the *Y*-chromosome from CBA together with control strains produced in the same way. Thus the comparison of the effects of the two *Y*-chromosomes could be made on two different backgrounds, CBA and C57, 'background' meaning the autosomes, the *X*-chromosome, and any maternal effects that there may have been (Hay, 1975). The mice used came from the 6th to 10th

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Table 1. *Designation of strains, backgrounds, and Y-chromosomes*

Source strains	Abbreviations		
	Background	*Y*-chromosome	
1. CBA/FaCam St	CBA	C	
2. C57BL/Fa St	C57	B	
3. PHL/St	PHL	L	
4. (PHL)-YH/St	(PHL)	H	
Crosses	**Background**	***Y*-chromosome**	**Abbreviations for congenic lines**
1. Repeated backcross to CBA of (CBA × C57) F1 male	CBA	B	CBA-YB
Repeated backcross to CBA of (C57 × CBA) F1 male	CBA	C	CBA-YC
2. Repeated backcross to C57 of (CBA × C57) F1 male	C57	B	C57-YB
Repeated backcross to C57 of (C57 × CBA) F1 male	C57	C	C57-YC
3. Backcross to PHL of (PHL × (PHL)-YH) F1 male	PHL	H	PHL-YH
Backcross to PHL of ((PHL)-YH × PHL) F1 male	PHL	L	PHL-YL
4. Backcross to CBA of PHL-YH male from 3. above	CBA/PHL	H	CBA/PHL-YH
Backcross to CBA of PHL-YL male from 3. above	CBA/PHL	L	CBA/PHL-YL
5. Backcross to C57 of PHL-YH male from 3. above	C57/PHL	H	C57/PHL-YH
Backcross to C57 of PHL-YL male from 3. above	C57/PHL	L	C57/PHL-YL

(PHL) denotes known lack of complete congenicity of the source PHL-YH strain with PHL (see text).

generation of backcrossing. The generations were consistent in respect of all the observations made.

The PHL strain was originally selected for low blood pH (Weir, 1953). Another strain, PHH, was selected for high blood pH. The PHL-YH strain was made by crossing males from PHH to females from PHL followed by repeated backcrossing to PHL, as described by Weir (1976). Thus the PHL and PHL-YH strains when obtained differed in their *Y*-chromosomes and were theoretically already congenic for background, except for a region around the tan belly locus used as a marker to distinguish the strains in the animal house. Additional backcrosses were made as shown on Table 1, backcross 4, in order to eliminate possible effects of the tan belly locus. These two strains provided a comparison of the *Y*-chromosomes from PHL and from PHH on the common background of PHL. In order to allow the comparison to be made on other backgrounds, males from PHL and from PHL-YH were each crossed to females from CBA and from C57, and the F1 males were used. This provided two additional backgrounds, both heterozygous, one being the CBA × PHL background and the other the C57 × PHL background.

Several of the lines were checked for congenicity by grafting tail skin from a female donor to a female host. In the case of the C57BL and CBA strains, skin from 10 C57-YC and C57-YB females in generations 7 and 8 was grafted to C57BL recipients. Only one graft, from a donor at generation 7, was rejected before 100 days. Males from these strains nevertheless showed significant differences in response to exogenous andro-

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gen (see below). In the case of the lines derived from the PHL and PHH strains, grafts were made from CBA/PHL-YH and CBA/PHL-YL female donors to CBA/PHL-YH and CBA/PHL-YL female hosts in all four combinations in equal numbers. None of the 24 grafts was rejected before the end of the observation period (100 days), but males from the PHL-YH and PHL-YL lines showed differences in testosterone levels (see below). Given the sensitivity of the grafting technique to small residual genetic differences, these results are satisfactory and indicate that the differences described below are virtually certain to be due to the different *Y*-chromosomes carried by the congenic strains, rather than to any autosomal differences between them.

The male progeny of the crosses were all 8 weeks old at the time of the experiments; all available animals were included as they reached that age.

(ii) *Radioimmunoassay of serum testosterone*

Testosterone was assayed according to Abraham (1974) with the following modifications. Male mouse serum was diluted (1:10) with phosphate buffer. Testosterone was extracted from 0·1 ml of the diluted samples with 0·1 ml diethyl ether. Testosterone was assayed by adding 0·1 ml of antiserum, followed by 0·1 ml of tritiated testosterone at 4 °C. The contents were mixed and equilibrated for (1½ h, at 4 °C. 0·1 ml of activated charcoal was added and centrifuged after 15 min at 1900 g for 10 min at 4 °C. The supernatant containing the antibody-bound testosterone was counted in 10 ml of scintillation fluid.

(iii) *Organ weights*

The animals were killed by cervical dislocation and the kidneys, the preputial and the seminal vesicles (blotted to remove stored secretions) were removed and weighed.

(iv) *Sensitivity of target-organs to exogenous androgen in castrated mice*

Four week old male mice were castrated under sodium pentobarbitone anaesthesia. Two weeks later, the mice from each line were divided into the following dose groups at random:

(*a*) castrated controls injected with olive oil only;

(*b*) castrates given 0·4, 2·3, 3·6 or 4·8 μg/g body weight of testosterone propionate (TP) in olive oil each alternate day for 2 weeks.

No significant differences in body weight between congenic pairs of mice were found ($P = 0{\cdot}5$), so this procedure introduced no bias between lines.

Each group received subcutaneous injections of 50 μl of the olive oil preparations between 10·00 and 12·00 h. The mice were killed 24 h after the last injection and the organs weighed. Intact, uninjected animals were also examined.

3. Results

(i) *Serum testosterone levels*

The results for serum testosterone levels are summarised in Table 2. A significant *Y*-chromosome effect on serum testosterone levels between the lines carrying the L and H *Y*-chromosomes on both the PHL (Fig. 1) and the C57 genetic backgrounds was revealed by the Mann–Whitney U-Test. It should particularly be noted that the *Y*-chromosomes from L and H affect the testosterone distributions in the same direction and in the same proportions on both the PHL and C57 genetic backgrounds; this difference is superimposed on the variation between the L and B backgrounds. However, no statistically significant difference was found between the L and H *Y*-chromosome lines on the CBA genetic background ($n = 78$ and 68).

No statistically significant effect on testosterone levels between mice with *Y*-chromosomes from strains CBA and C57 was seen on either genetic background (confirming the work of Stewart, Manning & Batty, 1980; $P > 0{\cdot}5$).

Genetic background itself had a large effect: serum testosterone levels for both the PHL-YH and PHL-YL lines were significantly higher than the rest of the lines examined.

(ii) *Androgen target organ weights of intact animals*

No significant *Y*-chromosome effects were observed between pairs of the congenic lines when testosterone-

Table 2. *A 2 × 2 contingency table for the mean serum testosterone levels (nmol/l) of two pairs of congenic lines, together with probability values of the Mann–Whitney U-test (2-tailed). The numbers of mice examined are given in parantheses*

		Y-Chromosome		
		L	H	(P)
Background	C57/PHL	1·28 (40)	0·90 (45)	< 0·05
	PHL	3·43 (30)	2·25 (22)	< 0·05
	(P)	< 0·001	< 0·001	

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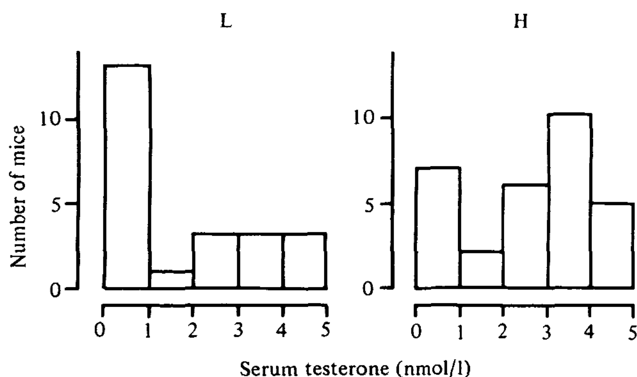

Fig. 1. Distributions of serum testosterone levels for L and H Y-chromosomes on a common PHL background (strains PHL-YL and PHL-YH).

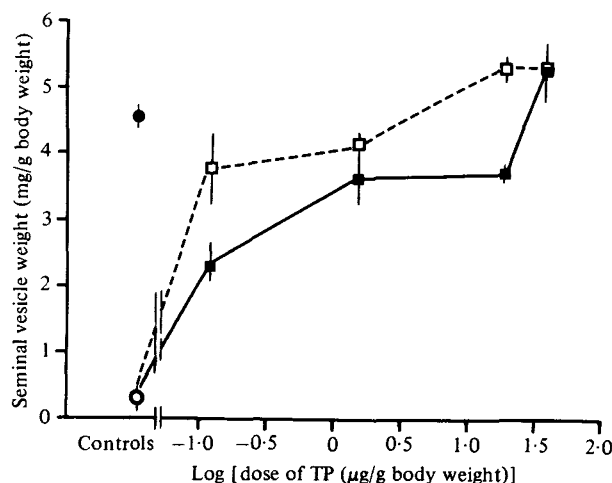

Fig. 2. Log-dose/response curves for seminal vesicle weights (and their standard errors) of castrated male mice of two different genotypes injected with exogenous testosterone propionate (TP) in oil. ■---■, C57-YB ($n = 22$); □---□, C57-YC ($n = 36$); ●, intact controls; ○, controls injected with oil only.

dependent target organs (namely the kidneys, the preputial or the seminal vesicle weights) were examined in intact animals (Table 3, $P = 0{\cdot}5$, using Student's t-tests). These results are consistent with the work of Hayward & Shire (1974) and Stewart *et al.* (1980) on the CBA strain. However, highly significant differences were observed between the different genetic backgrounds themselves.

(iii) *Castration and sensitivity to testosterone propionate*

Statistical comparisons were carried out at each of the various dose levels employed. On the C57 genetic background, a difference between the B and C Y-chromosomes was found for seminal vesicles at a dose of 3·6 μg TP/g body weight (Student's t-test, $P < 0{\cdot}001$). A consistent trend is seen at other dose levels but does not reach statistical significance (taken as $P < 0{\cdot}01$ because of the number of comparisons involved: see Fig. 2). Overall, there is good evidence for a Y-effect on the sensitivity of seminal vesicle to exogenous androgens.

A statistically significant Y-effect is also seen on kidney weight at a dose of 0·4 μg/gm ($P < 0{\cdot}001$, Fig. 3). This apparent effect on the sensitivity of the kidney must however be viewed with some reservation because it is dependent on the reality or otherwise of the rise in kidney weight observed in animals carrying the C Y-chromosome compared with oil injected controls of the same genotype ($P = 0{\cdot}01$), and because the effect is not consistent at other dose levels. Comparison of preputial weights at a dose of 1·2 μg/g also yields a significant difference (Fig. 4); but as with the kidney there is no consistent trend at other doses, and the significance level ($P < 0{\cdot}01$) is lower than for the seminal vesicle.

Further, no effects of Y-chromosomal origin were seen between the B and C Y-chromosomes on the CBA genetic background. There was no significant difference between mice with the L and H Y-chromosomes on either genetic background, although comparable numbers of mice were examined (lines C57/PHL-YL and C57/PHL-YH; CBA/PHL-YL and CBA/PHL-YH).

Table 3. *Summary of the organ weight data collected on the 10 lines of males* (*mean* ± S.E.)

Background	Y-chromosome	n	Organ weight (mg/g body weight) Preputial	Seminal vesicle	Kidney
CBA	B	161	2·2±0·1	4·0±0·1	14·7±0·2
CBA	C	133	2·0±0·1	3·8±0·1	14·6±0·2
C57	B	84	1·8±0·1	4·4±0·1	14·5±1·4
C57	C	56	1·9±0·1	4·5±0·1	13·3±0·4
PHL	H	41	3·9±0·2	3·8±0·2	14·4±0·3
PHL	L	27	3·5±0·3	3·3±0·1	14·3±0·2
CBA/PHL	H	195	2·8±0·1	4·1±0·1	14·1±0·3
CBA/PHL	L	213	2·8±0·1	4·1±0·1	15·4±0·2
C57/PHL	H	85	3·7±0·1	4·5±0·1	13·8±0·2
C57/PHL	L	70	3·8±0·1	4·6±0·1	14·0±0·2

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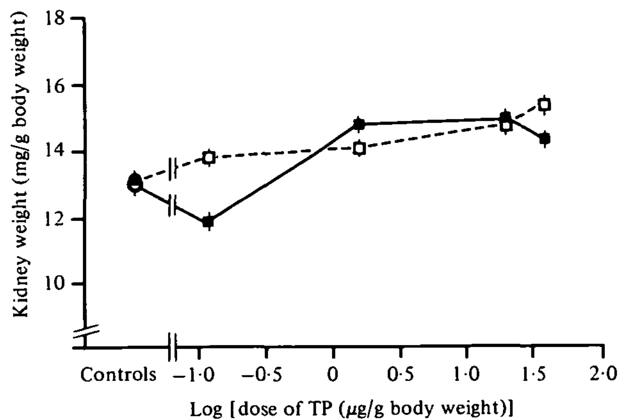

Fig. 3. Log dose/response curves for kidney weights, and their standard errors (symbols as in Fig. 2).

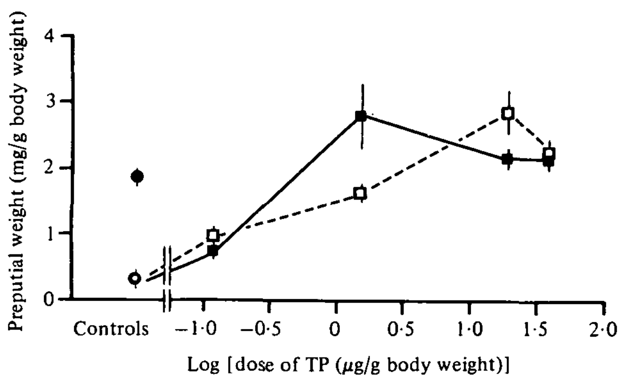

Fig. 4. Log dose/response curves for preputial weights and their standard errors (symbols as in Fig. 2).

4. Discussion

The results presented here provide strong initial evidence that alleles on the *Y*-chromosome vary both serum testosterone levels and the sensitivity of certain target organs to exogenous androgen. In each case, there is evidence that the genetic background in which the *Y*-chromosomes are operating modulates the phenotypic expression of the allelic differences. For example, the *Y*-linked alleles derived from strains PHL and PHH are seen to have the same proportional effect on serum testosterone levels on the PHL and C57 backgrounds, but no effect when placed on the CBA background. Similar interactions with genetic background of the *Y*-linked action on spermatogenesis have also been observed (unpublished observations). The differences between the congenic lines are very unlikely to be due to autosomal rather than *Y*-chromosome differences, as the relevant strains were shown to be satisfactorily homogeneous by appropriate skin grafts.

It must be emphasized that the *Y*-chromosome pairs chosen for study were those where previous reports had indicated the existence of allelic *Y*-chromosomal variation affecting other characters (Hayward & Shire, 1974; Weir, 1976; Stewart *et al.* 1980). In particular, it appears that other CBA strains do not show the *Y*-effect on testis weight characteristic of CBA/FaCam (unpublished observations).

Selmanoff *et al.* (1977) measured plasma testosterone levels in C57BL/10, DBA/1 and reciprocal F1 hybrid mice and found that there was a *Y*-effect on the rate of change in androgen levels during puberty. This has been disputed, both because the *Y*-chromosome effects were confounded with possible maternal and *X*-chromosomal effects, and on statistical grounds (Hay 1975; Stewart *et al.* 1980). The present study is therefore the first to provide clear evidence of a *Y*-chromosomal effect on serum testosterone levels. In this case, the variant *Y*-chromosomes were derived from the lines PHL and PHH (Weir, 1953, 1976). The CBA and C57 *Y*-chromosomes show no such effect, even on a similar genetic background. This report includes all results published in preliminary form (Jutley & Stewart, 1981, 1984; Stewart, 1983) as well as additional results not previously available.

The distribution pattern of testosterone within the PHL-background genotypes appear to be bimodal (Fig. 1), which is compatible with the known episodic nature of testosterone release in the mouse (Bartke *et al.* 1973; 1975). It is particularly significant that the same difference in distributions, with the effect of the *Y*-chromosome substitution operating in the same direction, was observed independently on the CBA genetic background. On this interpretation, the effect of *Y*-chromosome substitution is to vary the frequency of the pulsatile release of testosterone (the PHH *Y*-chromosome increasing it compared with that from PHL), since the difference between the congenic lines is in the relative numbers of animals in each part of the bimodal distribution rather than in the overall range of testosterone values. This would suggest that the primary effect of the *Y*-chromosome is at the hypothalamic/pituitary level, but it would be necessary to examine the relevant peptide hormones to confirm this suggestion.

This study also provides evidence for alleles on the *Y*-chromosome affecting sensitivity to androgens of target organs, most particularly seminal vesicle. This effect was only seen when the CBA and C57BL *Y*-chromosomes were substituted for each other (not with the PHH and PHL *Y*-chromosomes) and was confined to the C57BL/PHL genetic background. Nothing is known of the way in which this effect is produced.

When the results are collated, an apparent paradox results. Why do the weights of androgen-sensitive target organs not vary with *Y*-chromosomal origin in intact animals (between C57BL and CBA because of differences in target organ sensitivity; between PHH and PHL because of variation in androgen levels)? The answer may lie in the relation of the dose-response curves to the naturally occurring blood levels. For example, all organs in the C57BL and CBA congenic castrates required a dose of 1·2 μg/g body weight of testosterone propionate every other day to maintain

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organ weights equivalent to the intact controls, which exceeds the dose at which the *Y*-effect can be seen. The endogenous testosterone levels are consequently likely to be above the dose range at which a *Y*-effect on organ weight could be seen in intact animals.

The question arises as to how many separate effects of the *Y*-chromosome can be defined from these comparisons. The effects on serum testosterone levels and seminal vesicle sensitivity must be regarded as distinct, since each is seen in the absence of the other even when the two pairs of *Y*-chromosomes are placed on similar genetic backgrounds. Therefore, a minimum of two separate loci on the *Y*-chromosome must be postulated to explain these observations. However, the relation of each of these effects to others defined for the same *Y*-chromosome pairs (such as testis weight, and aggressive intermale behaviour for CBA and C57BL; and perhaps sex ratio of offspring for PHH and PHL) requires further analysis (Stewart, 1983). From the pattern of results observed in the various strains, the effects on testis development and androgen sensitivity have not so far been separated, but the effect on testosterone levels is not associated with any of these other actions of the *Y*-chromosome. Stewart (1983), reviewing the literature, concluded that there was evidence for separate effects of mouse *Y*-chromosomes on testicular growth and development, spermatogenesis, and aggressive behaviour, in addition to the effects described in this study. The relationship between these actions of the *Y*-chromosome and the number of loci which produce them will be the subject of further reports. Rapid progress in the molecular analysis of the mammalian *Y*-chromosome (see for example Goodfellow *et al.* 1983; Erickson & Goodfellow, 1984) may also be expected to throw light on this problem.

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