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Effects of *Y*-chromosome variants on the male behaviour of the mouse *Mus musculus*

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SUMMARY

A study of crosses between CBA/FaCam and C57Bl/6Fa mice revealed an effect of the origin of the *Y*-chromosome on testis weight and aggressive behaviour, but failed to reveal any effect on sexual behaviour and androgen metabolism. There is therefore no evidence that androgens mediate the *Y*-linked variation in aggressive behaviour and testis weight. On behavioural grounds, it is difficult to compare measures of sexual and aggressive behaviour, but it appears from these results that there are major genetic components on the *Y*-chromosome controlling the development of sexual and aggressive behaviour which are distinct.

1. INTRODUCTION

Although few genetic factors have been identified on the *Y*-chromosome there are two reports in the literature of *Y*-chromosome effects on the behaviour of male mice. Weir & Hogle (1973) and Weir (1976) have reported an effect of variant *Y*-chromosomes on sexual behaviour but their behavioural measures were inadequate for a real appraisal. Selmanoff *et al.* (1975) reported a *Y*-linked effect on the aggressive behaviour of DBA/1 and C57Bl/10 strains. Their conclusions were criticised by Hay (1976), who pointed out that *Y*-chromosome effects were confounded with possible maternal effects in their reciprocal F_1 hybrids. A recent study by Maxson, Ginsburg & Trattner (1979) overcomes this objection by using a strain produced by repeated backcrossing whose males carry C57Bl/10 autosomes combined with a DBA/1 *Y*. They confirmed that the origin of the *Y*-chromosome does have a marked effect on levels of aggression. However, the measure used in all their tests was not really satisfactory for it involved pairing males within strains only. Thus the stimulus value of each strain was compounded with the level of its aggressive responsiveness.

The purpose of this study, a preliminary account of which has already been published (Stewart, Manning & Batty, 1978), is to examine further the effects of the *Y*-chromosome using more complete behavioural measures in stocks so bred

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that it is possible to isolate the inheritance of the *Y*-chromosome from the genotype of the mother. For this purpose we used two strains, C57Bl/6 and CBA, which are sufficiently contrasted behaviourally and whose *Y*-chromosomes are known to exhibit an effect on testis weight (Hayward & Shire, 1974). It is possible that some behavioural effects could be mediated by variation in androgen secretion or metabolism, and we also measured serum testosterone levels and the weight of organs known to be testosterone-dependent.

2. MATERIALS AND METHODS

All mice were bred in the Department of Biological Sciences, University of Lancaster. The animals were maintained at 20 ± 2 °C on a reversed lighting schedule (12 h light, 12 h dark) throughout the year, and fed on the FFG(LAC) mouse diet (Dixon's). The young mice were weaned at $3\frac{1}{2}$ weeks of age, sexes separate, at a density of 5–8 animals per cage.

The strains used were derived from reciprocal crosses between CBA/FaCam and C57Bl6Fa according to the following programme (female first):

(1) CBA × C57Bl
C57Bl × CBA

(2) CBA × (CBA × C57Bl) = B_1(CBA) (Y-C57Bl)
CBA × (C57Bl × CBA) = B_1(CBA) (Y-CBA)
C57Bl × (CBA × C57Bl) = B_1(C57Bl) (Y-C57Bl)
C57Bl × (C57Bl × CBA) = B_1(C57Bl) (Y-CBA)

(3) CBA × B_1(CBA) (Y-C57Bl) = B_2(CBA) (Y-C57Bl) (designated 'A')
CBA × B_1(CBA) (Y-CBA) = B_2(CBA) (Y-CBA) (designated 'B')
C57Bl × B_1(C57Bl) (Y-C57Bl) = B_2(C57Bl) (Y-C57Bl) (designated 'C')
C57Bl × B_1(C57Bl) (Y-CBA) = B_2(C57Bl) (Y-CBA) (designated 'D')

Although some autosomal segregation would still be occurring within each of these lines, the pair of lines A and B (and likewise C and D) would differ systematically from each other only in the origin of the *Y*-chromosome. Each animal within these pairs will carry the same *X*-chromosome, the same autosomal alleles (on average) and will have been subjected to the same maternal and cytoplasmic effects. As far as their chromosomes are concerned we may summarize that A has largely CBA autosomes and *X*, with C57 *Y*; B has CBA autosomes and *X*, with CBA *Y*; C has C57 autosomes and *X*, with C57 *Y*, and D has C57 autosomes and *X* with CBA *Y*. All male animals from these four lines which were born over a 7-week period were collected, identified solely by a code number, and transported to Edinburgh where all further work was conducted 'blind'. Males were a minimum of 10 weeks old at the start of testing and there was no significant difference between the four lines in the age distribution of the mice. A total of 49 animals were tested, and all except one completed the testing programme. In Edinburgh, the animals were caged individually in clear plastic cages, $33 \times 16 \times 12$ cm, 10 days before behavioural testing began, under reversed lighting (on at 8.00 p.m., off

at 8.00 a.m.). All tests were carried out between 9.00 a.m. and 1.00 p.m., and each test was repeated twice weekly (aggressive behaviour, Monday and Thursday, sexual behaviour, Tuesday and Friday) to a total of five tests for each behaviour with each animal.

(i) *Aggression tests*

We used a modified version of the 'standard opponent' test whose advantages have been cogently argued by Brain & Poole (1974). Intact BALB/c strain males, which had previously been defeated in aggressive encounters and did not fight back, were used as stimulus animals. One such male was put into the home cage of each male and the pair were then observed for 15 min unless fighting occurred sooner. The latency to fighting was recorded and mounting of the stimulus animal was noted if it occurred. Fighting was defined as the experimental animal attacking the stimulus animal so as to cause it to squeak and rear up defensively. The pair were separated as soon as possible following such an attack.

This measure of aggression relies simply on the occurrence and latency to the onset of fighting rather than using observation of fighting behaviour itself. Brain & Poole (1974, 1976) also included measures of 'accumulated attacking time' (AAT) and number of attacks per test. We did not choose to let fighting develop, but Brain and Poole's studies show that there is a clear negative relationship between AAT and latency to the first attack. Hence latency is probably a good measure of fighting tendency.

(ii) *Sexual behaviour*

Sexual behaviour towards a BALB/c female, brought into an artificial oestrous condition by previous oestrogen administration, was tested using the methods described by McGill & Manning (1976). The behaviour recorded was the occurrence and the latency of first mounting, of first intromission, and of ejaculation following the establishment of intromission. Tests were terminated after 30 min. if no sexual behaviour beyond mounting has been observed, and after 2 h if ejaculation had not occurred following intromission.

(iii) *Other measurements*

On completion of the behavioural tests, the animals were weighed. A blood sample was taken and plasma testosterone determined by radioimmunoassay as described by Batty (1978). The testes, seminal vesicles (blotted to remove stored secretions) and kidneys were weighed.

3. RESULTS

For the behavioural tests, a mean score for each animal and parameter was calculated from the last four of the five tests. For the purpose of calculating these averages, nominal latency values of 20 min in the aggression test and 40 min in the sex test were ascribed to an animal not displaying the behaviour in question during the test.

There were no significant Y-chromosome effects on body weight, but testis weight differed significantly between lines A and B and between C and D (Table 1; $P < 0{\cdot}01$ by analysis of variance). The Y-chromosome from CBA lowered testis weight by 25% on the CBA background and 29% on the C57 background compared with the Y-chromosome from C57Bec. There were no significant Y-chromosome effects for seminal vesicle weight, kidney weight or plasma testosterone levels (Table 1). The behavioural data are given in Table 2. For sexual behaviour

Table 1. *Summary of the physical data collected on the four groups of males*

(Mean ± S.E. in each case)

Group	N	Testis wt (mg)	Plasma testosterone (ng/ml)	Seminal vesicle wt (mg)	Kidney wt (mg)
A	8	152·50 ± 9·49	3·62 ± 1·17	80·00 ± 9·11	453·67 ± 45·56
B	14	113·50 ± 2·97	4·27 ± 0·77	89·43 ± 5·10	406·36 ± 12·39
C	15	194·27 ± 4·32	2·67 ± 0·71	104·87 ± 5·33	333·87 ± 9·46
D	12	128·17 ± 7·34	2·35 ± 0·57	98·93 ± 5·72	315·50 ± 11·01

Table 2. *Summary of the behavioural data collected on the four groups*

	Group			
	A	B	C	D
N	8	14	15	12
Mean no./5 tests				
Mounting	3·57 ± 0·43	3·79 ± 0·42	4·80 ± 0·14	4·83 ± 0·17
Intromitting	1·43 ± 0·72	1·86 ± 0·52	4·53 ± 0·22	4·42 ± 0·34
Ejaculating	0·43 ± 0·30	1·14 ± 0·47	4·00 ± 0·32	3·67 ± 0·48
Mean min.				
Mount latency	17·27 ± 3·02	16·60 ± 2·01	4·95 ± 0·99	4·11 ± 1·06
Intromission latency	30·53 ± 2·80	28·20 ± 2·11	8·84 ± 1·36	6·20 ± 1·46
Mean no. fighting/5 tests	4·43 ± 0·30	4·28 ± 0·32	3·00 ± 0·61	4·75 ± 0·13
Fight latency (mean min.)	1·98 ± 0·96	3·24 ± 0·82	8·94 ± 1·19	1·02 ± 0·41

there are highly significant differences in all the measures between the lines (A and B) with CBA autosomes and X and those (C and D) with C57 autosomes and X. C57 mice mounted and intromitted females more often and with lower latencies and ejaculated more often. Such quantitative differences between inbred strains are familiar enough from the work of McGill (1962) and others. It is more relevant to this study to note that we found no effect on sexual behaviour relating to the Y-chromosome.

There were such effects on aggressive behaviour (Table 2). On the C57 background (lines C and D) there was a Y-effect on the proportion of animals fighting; 5/15 animals from line C whose Y-chromosome was also derived from C57 did not fight at all, whereas all 12 animals from line D (CBA Y-chromosome) did fight. This result is of marginal statistical significance since the groups are small, but testing the hypothesis that D-line males fought sooner than those from C

gave a highly significant result (expressed as frequency histograms in Fig. 1). The data were transformed logarithmically and then one-one-tailed Student's $t_{20} = 4{\cdot}7$, ($P < 0{\cdot}001$). In addition, mice of line C displayed more mounting behaviour in the aggression test than line D (9/15 animals mounted, as against 2/12). This is an independent result, as some animals both mounted and fought, and would have a probability of $P < 0{\cdot}05$ on the binomial distribution if there were no difference between the lines. On the CBA background, all but two animals

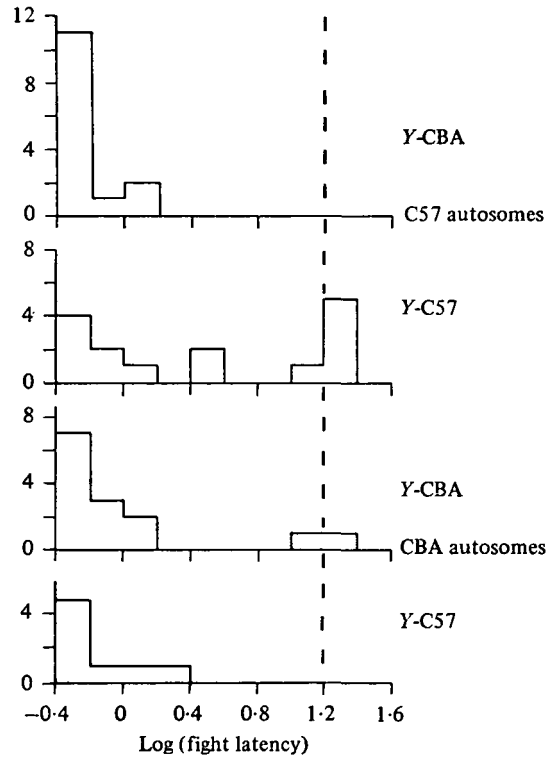

Fig. 1. The latencies to fight (log min) of the four groups of males. The dotted line marks 15 min, the cut-off point in the tests.

fought with extreme rapidity on most occasions, and there were no statistically significant *Y*-chromosome effects. In summary, animals with the *Y*-chromosome from CBA showed decreased testis weight and increased aggressive behaviour. No significant effects on sexual behaviour or androgen metabolism were detected.

4. DISCUSSION

Our results confirm the findings that variant *Y*-chromosomes could produce variation in testis weight. (Hayward & Shire, 1974) and aggressive behaviour (Maxson, Ginsburg & Trattner, 1979). On the other hand, they failed to provide evidence for a *Y*-effect on sexual behaviour, androgen secretion or androgen metabolism.

Before the relationship between these various characters is discussed, it is necessary to mention some of the inherent shortcomings of this type of pleiotropic study. Unfortunately different loci on the *Y*-chromosome cannot be separated by recombination analysis. Furthermore, the inability to demonstrate a *Y*-effect does not mean that it is absent, for it may be expressed only on a particular genetic background. Whereas the *Y*-effect on testis weight is robust with respect to genetic background, this is not true of the *Y*-effect on spermatozoan abnormality reported by Krzanowska (1972), nor perhaps of the *Y*-effect on aggressive behaviour described in this paper. There could be a 'ceiling effect' operating with the CBA background; CBA autosomes may render males so aggressive that *Y*-chromosome effects were not detected. Furthermore, whereas testis weight is a clearly defined character, our measurements of sexual behaviour and aggressive behaviour differ from those of previous studies and may relate to different characters (or components of characters) from those studied by Weir (1976) and Selmanoff *et al.* (1975). We chose to use well-established behavioural tests; the test of sexual behaviour, in particular, is fairly comprehensive (McGill & Manning, 1976).

Within these limitations, our study reveals some degree of dissociation between the patterns of inheritance of sexual and aggressive behaviour of male mice; this is not surprising. Although it is desirable to have further confirmation of an effect of *Y*-linked factors on sexual behaviour (Weir, 1976), it would seem possible that there are at least two separate developmental effects ('loci') of the *Y*-chromosome; one affecting testis weight and aggression and one affecting sexual behaviour. This suggestion is consistent with the literature. *Y*-effects on testis weight and aggression have yet to be dissociated, as Herrick & Wolfe (1977) have reported that DBA and C57 strains have *Y*-variants affecting testis weight as well as aggression (Selmanoff *et al.* 1975). In PHH and PHL mice, where there may be a *Y*-effect on sexual behaviour (Weir, 1976), Herrick and Wolfe did not find any *Y*-linked variation in testis weight. *Y*-linked effects on spermatozoan abnormality (Krzanowska, 1972) and sex ratio (Weir, 1976) may be due to further factors, but their relationships with those just described require more investigation.

Although the occurrence of male aggressive behaviour is determined by the presence of androgens secreted first in the neonate and then again following puberty, there is no evidence from this study, nor from a very large body of data on seminal vesicle and kidney weights (Hayward & Shire, 1974; Stewart, unpublished), that variation in androgen secretion or metabolism is responsible for mediating the *Y*-linked variation in aggressive behaviour or testis weight. Selmanoff *et al.* (1977) measured plasma testosterone levels in C57Bl/10, DBA/1 and reciprocal F_1 hybrids through the first 2 months of life. Although they claim evidence for a *Y*-effect on the rate of change of androgen levels over puberty, their data are unconvincing and such differences as are found could be due to autosomal loci. Batty (1978) also measured plasma testosterone levels in adult mice of C57Bl/6, DBA/2 and their hybrids and found no significant difference between the reciprocal F_1 males. It is still possible that there is variation in androgen

secretion before puberty or variation in the sensitivity of both testis and hypothalmus (but not of the seminal vesicle and kidney) to androgen, but it seems much more likely that the *Y*-linked effects are mediated by some other mechanism. For example, the apparently continuing effect on testis weight from birth onwards (Hayward & Shire 1974) would be consistent with a possible action of the H-Y antigen (Ohno *et al.* 1976), which is present in all male tissues from an early stage.

Whatever the mechanisms involved, from the behavioural point of view these results provide some further evidence that the mechanisms controlling aggressive and sexual behaviour are distinct and suggest that their development involves some genetic components unique to each. It is important to emphasize that such components controlling male behaviour are not confined to the *Y*-chromosome. Lagerspetz's (1964) successful selection for high and low levels of aggression in mice has demonstrated how much additive variation is available in mouse populations. The study of Maxson *et al.* (1979), together with our own results show that the autosomes of different strains vary greatly in genes affecting aggressive and sexual behaviour.

Although the effects of the *Y*-chromosome on male behaviour are of continuing interest in view of the possible effects of supernumary *Y*-chromosomes on human behaviour (Meyer-Bahlburg, 1974; Dorus, 1978) due caution must be exercised in extrapolation between species. Variant *Y*-chromosomes could account for some of the differences between human *XYY* males but environmental and cultural variables are certain to have major effects on the development of human aggressive behaviour.

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