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Paternal inheritance of egg traits in mice: a case of genomic imprinting

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Summary

Eggs from reciprocal hybrids between the C57BL/6By and BALB/cBy strains were tested for their susceptibility to attack by hyaluronidase and pronase. There were significant reciprocal differences between the F_1 females in the responses of their unfertilized eggs to both enzymes. The F_1 hybrids from BALB mothers showed the increased susceptibility characteristic of C57BL whilst the F_1 hybrids with C57BL mothers were more resistant to both enzymes, like BALB mice. Eggs from the four kinds of reciprocal F_2 hybrid females also showed patroclinous patterns of susceptibility. A patroclinous difference was found between reciprocal crosses of the CXBD and CXBE recombinant inbred strains but not in crosses between recombinant inbred strains with similar phenotypes. Cross fostering did not alter the phenotypes of the C57BL and BALB females or those of their reciprocal F_1 hybrids. The findings are interpreted in terms of differential genomic imprinting of paternally inherited information. The possible general usefulness of patroclinous differences between reciprocal F_1 females in revealing differences in imprinting is noted.

1. Introduction

In mammals the two parental genomes are not functionally equivalent during embryogenesis, for both maternal and paternal genomes are required for normal development (Davor & Solter, 1984; Surani *et al.* 1984, 1987). In mice specific chromosomal regions that require to be transmitted from both mother and father to the zygote to ensure normal embryonic and neonatal development have been identified using Robertsonian translocations. Parts of chromosomes 2, 6, 7, 8 and 17 have been shown to be involved (Lyon, 1983; Cattanach & Kirk, 1985; Searle & Beechey, 1985) and a map of affected and unaffected regions produced (Beechey *et al.* 1988). Similarly an inequality of parental contributions has been shown for the mutation hairpin-tail (T^{hp}). This causes embryonic lethality in the heterozygote when the mutation is contributed by the mother, but not when inherited from the father (McGrath & Solter, 1984*a*; Johnson, 1975). It has been suggested that only the maternal allele is in a state that can be activated during development and that the paternal allele is held in an inactive state by genomic imprinting. Differential silencing has also been proposed to account for variation in the phenotypic expression of the Fused (*Fu*) mutation (Belyaev *et al.* 1981; Ruvinsky, 1988), which is also on chromosome 17.

Detectable differences between inbred strains would be expected in the strength or occurrence of genomic imprinting, at least for loci that were not essential for viability. For traits differing between strains this would result in differences between the F_1 mice produced by reciprocal crosses between the strains. Such reciprocal differences would be most easily identified in characters expressed in females, since F_1 females share identical complements of both autosomes and *X* chromosomes. We report here such a pattern of differences between F_1 females in two characters expressed only in females. These characters are the susceptibility of the cumulus oophorus and the zona pellucida surrounding the egg to enzymic attack by hyaluronidase and protease. The eggs of C57BL mice, of several sublines, are known to be relatively susceptible to enzymic attack *in vitro* (Braden, 1958; Krzanowska, 1972; Nicol & McLaren, 1974; Bander *et al.* 1988). Oocytes from BALB/c mice are more resistant to both hyaluronidase and pronase, as are

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eggs from some of the CXB recombinant inbred strains (Bander *et al.* 1988).

This paper describes the phenotypes of eggs from females from the two reciprocal F_1 crosses and four reciprocal F_2 crosses between C57BL/6By and BALB/cBy, together with the results of a cross-fostering experiment. Measurements on reciprocal F_1 crosses involving some of the CXB recombinant inbred strains are also presented.

2. Materials and Methods

(i) *Mice*

Eggs were obtained from adult female mice, aged from 10 to 12 weeks, bred at the University of Essex. Mice from the C57BL/6ByEss and BALB/cByEss inbred strains were crossed reciprocally to produce F_1 and F_2 hybrids. In describing the hybrids the maternal parent is given first. C57BL is abbreviated to B and BABL/c to C. There were two reciprocal F_1 crosses (C × B and B × C) and four reciprocal F_2 crosses: (C × B) × (C × B), (C × B) × (B × C), (B × C) × (C × B) and (B × C) × (B × C). In addition measurements were made on reciprocal crosses using the CXB recombinant inbred strains, CXBD/ByEss, CXBE/ByEss and CXBJ/ByEss. All mice were raised under standard environmental conditions (Shukri *et al.* 1988). Some mice were cross fostered at birth by exchanging litters between mothers of different genotype.

(ii) *Measurements on eggs*

Ovulation was induced by injections of 8 i.u. pregnant mare gonadotrophin (PMSG: Sigma Chemical Co., St Louis, U.S.A.) followed by 8 i.u. of human chorionic gonadotrophin (hCG: Boehringer-Mannheim Gmbh, Mannheim, F.R.G.). Eggs in cumulus were removed from oviducts 18 h after hCG and transferred to 0·5 ml of phosphate-buffered saline (PBS) containing 12 i.u. bovine testicular hyaluronidase (Sigma) at 37 °C. Each clutch of eggs was observed every minute until all eggs were free of cumulus. Clutches of cumulus-free eggs were transferred to 0·5 ml of PBS containing 5 i.u. of pronase (Boehringer-Mannheim) at 37 °C. Each clutch of eggs was observed every minute until all eggs had lost their zona pellucida. All observations were made on clutches of at least 6 eggs from the same oviduct. The median dissolution time was calculated for each clutch of eggs for each enzyme treatment. The mean and standard error of the median dissolution time were calculated for each treatment for each genotype. Student's *t* tests were carried out with the SPSS package (Nie *et al.* 1975). Further details of the procedures are given by Bander *et al.* (1988).

3. Results

A preliminary investigation, at ambient temperature, of eggs from reciprocal crosses between C57BL and BALB showed them to differ significantly in susceptibility of their cumulus cells to dispersion by hyaluronidase. Measurements at 37 °C confirmed the difference in hyaluronidase susceptibility and demonstrated a significant reciprocal difference in the time required for pronase to digest the zona pellucida. Table 1 shows that, for hyaluronidase, the eggs from C × B F_1 females closely resembled the phenotype of their paternal strain as did those of the reciprocal B × C F_1 females. Susceptibility to pronase showed a similar pattern. Eggs from the reciprocal F_1 females differed significantly. Eggs from both the more and less susceptible F_1 females were freed from their zonae about two minutes sooner than eggs from the corresponding paternal strain. The F_2 crosses on the same side of the Table 1 are those with the same father but different mothers; those on the same line have different fathers but the same mother. F_2 crosses with

Table 1. *The mean times to disperse the periovum layers with hyaluronidase and pronase in C57BL and BAB mice and their reciprocal F_1 and F_2 hybrids*

Stock	*n*	Mean ± S.E. (min)	Stock	Mean ± S.E. (min)	*n*
		Hyaluronidase			
Inbred C57BL(B)	18	20·8 ± 0·30	BALB(C)	31·2 ± 0·32	27
F_1 C × B	30	19·6 ± 0·19	B × C	32·4 ± 0·42	28
F_2 (C × B) × (C × B)	56	20·0 ± 0·14	(C × B) × (B × C)	31·6 ± 0·29	36
F_2 (B × C) × (C × B)	53	19·9 ± 0·14	(B × C) × (B × C)	30·9 ± 0·36	52
		Pronase			
Inbred C57BL(B)	17	13·5 ± 0·45	BALB(C)	17·6 ± 0·43	20
F_1 C × B	28	11·1 ± 0·28	B × C	15·8 ± 0·24	25
F_2 (C × B) × (C × B)	53	12·4 ± 0·14	(C × B) × (B × C)	16·9 ± 0·21	36
F_2 (B × C) × (C × B)	49	12·8 ± 0·17	(B × C) × (B × C)	14·9 ± 0·19	48

n, number of clutches.
All comparisons on the same line were significantly different ($P < 0·001$).

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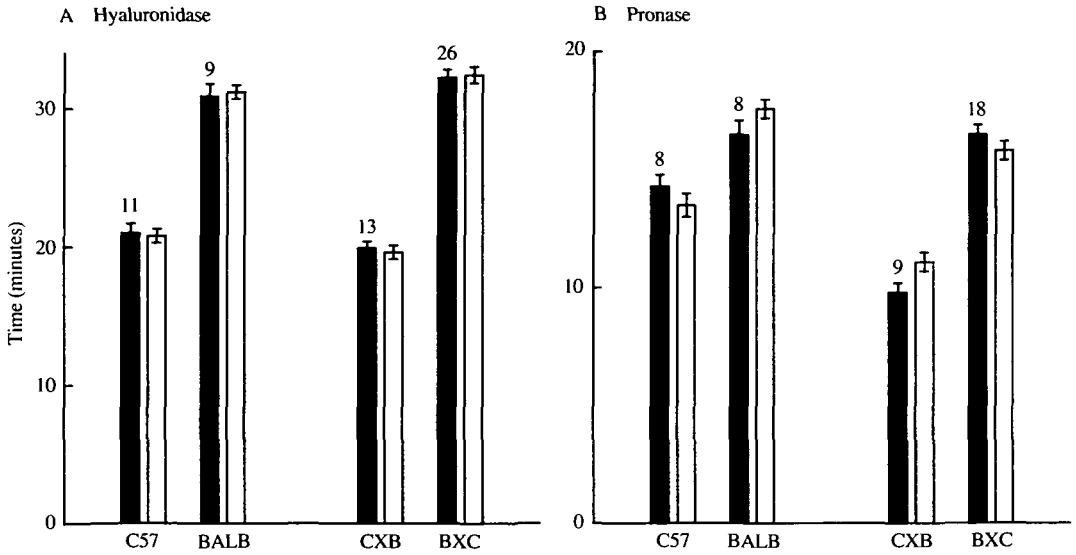

Fig. 1. The mean times (±S.E.) to disperse the periovum layers of eggs from crossfostered mice with hyaluronidase (*a*) and pronase (*b*). In each pair of columns, the filled column represents the eggs from mice crossfostered onto mothers of the other strain and the open column represents the control data (from Table 1) for the eggs from mice of the same genotype that remained with their natural mothers. The number above each column shows the number of clutches of eggs measured.

the same mother differed significantly for both enzymes. The mean hyaluronidase times for the F_2 crosses closely resembled those of their paternal and grand paternal stocks. The pronase times for F_2 crosses with C × B fathers were similar but those with B × C fathers differed significantly ($P < 0{\cdot}01$), but both had significantly higher values than any of the stocks in the C57BL paternal lineage.

Eggs were obtained from female mice that had been cross fostered between the parental strains or between reciprocal F_1 matings. The mean values for the enzymic dissolution of the periovum layers of their eggs are shown in Fig. 1. There were no significant differences in susceptibility to either enzyme between cross-fostered and non-fostered females of either strain or F_1 genotype.

Table 2 shows the mean times for hyaluronidase to disperse the cumulus cells of eggs from females from three sets of reciprocal F_1 crosses involving CXB recombinant inbred strains. The recombinant inbred strains included one with a short hyaluronidase time, CXBD, and two with long hyaluronidase times, CXBE and CXBJ (Bander *et al.* 1988). The eggs from both kinds of reciprocal F_1 female from C57BL × CXBD crosses did not differ significantly from each other or from the phenotypes of either parent, both of which had eggs that were very susceptible to the enzyme. Similarly the cumulus surrounding eggs from reciprocal crosses between two recombinant strains, CXBE and CXBJ, that each had very resistant cumulus oophori, were equally resistant to hyaluronidase at 37 °C. In contrast, the mean value for the CXBE × CXBD F_1 females was similar to that of their paternal strain whilst that of the reciprocal CXBD × CXBE F_1 females was significantly ($P < 0{\cdot}001$) greater.

4. Discussion

The significant differences between the phenotypes of the C57BL × BALB F_1 females rule out autosomal and sex-linked differences whilst the direction of the parental effect eliminates cytoplasmic or mitochondrial differences. Table 3 shows the genotypes (or, more strictly, the 'informotypes') predicted for hybrids under different patterns of inheritance. The observations on the F_1 hybrids are compatible with either a negative maternal effect or a positive paternal

Table 2. *The mean times to disperse the cumulus with hyaluronidase in crosses of CXB recombinant inbred mice*

Stock	Mean ± S.E. (min)	*n*
C57BL	20·8 ± 0·30	18
C57BL × CXBD	20·7 ± 0·33	27
CXBD × C57BL	19·1 ± 0·33	13
CXBD	21·2 ± 0·24	26
CXBE × CXBD	23·4 ± 0·45	27
CXBD × CXBE	29·7 ± 0·47	29
CXBE	46·8 ± 0·44	19
CXBE × CXBJ	49·9 ± 0·37	26
CXBJ × CXBE	48·4 ± 0·63	17
CXBJ	51·4 ± 0·48	23

Values for the three recombinant lines from Bander *et al.* (1988).
n, number of clutches.

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Table 3. *Sources of inherited variation contributing to the phenotype of female C57BL, BALB, F_1 and F_2 mice*

Stock	Autosomes	*X* chromosomes	Maternal	Negative Maternal	Paternal
C57BL	B	BB	B	(C)	B
BALB	C	CC	C	(B)	C
C × B F_1	Heterozygous BC	BC	C	B	B
B × C F_1	Heterozygous BC	BC	B	C	C
(C × B) × (C × B) F_2	Segregating B & C	BC & CC	C	B	B
(C × B) × (B × C) F_2	Segregating B & C	BB & BC	C	B	C
(B × C) × (C × B) F_2	Segregating B & C	BC & CC	B	C	B
(B × C) × (B × C) F_2	Segregating B & C	BB & BC	B	C	C

B represents the contribution from C57BL and C that from BALB/c. All the reciprocal F_2 crosses segregate for equal mixtures of two *X*-chromosome genotypes.

effect. *Y*-linked genes cannot be involved because the phenotype is sex-limited to females. No other factor seems to be involved for hyaluronidase susceptibility but the presence of a consistent shift in pronase sensitivity suggests that conventional nuclear factors might also contribute to the variation. Both negative maternal and positive paternal effects predict that the phenotypes of reciprocal F_2 hybrids would fall into two groups, each resembling one set of parents. However, the individual F_2 crosses that resemble each other are different for the two models (Table 3). The F_2 results for both enzymes did fall into two equal groups. In all cases pairs of F_2 crosses with a common maternal component differed significantly whilst the pairs of F_2 crosses sharing the same kind of father had similar means. Thus a negative maternal effect can be ruled out. The variances of reciprocal F_1 females were similar to the variances of the parental strains and also to the variances of the four kinds of F_2 cross. Taken as a whole the F_2 generation shows an increased overall variance, but only because of the large differences in mean values *between* the two groups of F_2 crosses. This contrasts with characters showing Mendelian inheritance, where increased variances are found *within* each of the four equivalent F_2 crosses.

The hyaluronidase sensitivity of the reciprocal crosses between inbred strains with similar phenotypes, whether susceptible (C57BL × CXBD) or very resistant (CXBE × CXBJ), did not discriminate between the models, as all predict that they would only, at least in some circumstances, reproduce the common parental phenotype. The reciprocal F_1 females from the cross of the CXBE and CXBD lines did differ significantly, and in the direction predicted by patroclinous inheritance. The mean for the CXBD × CXBE F_1 females was much closer to that of their BALB progenitor strain (Table 1) than to their immediate paternal strain.

The cross fostering experiment shows that the phenotypic differences were established before birth. There are two possibilities for the establishment before birth of the observed paternal effect. Either the father must influence the embryos' uterine environment in a way that produces lasting effects on the gonads of the offspring or the informational state of the paternal gametic contribution must be able to override the corresponding maternal one. We can conceive of no mechanism to mediate a consistent paternal uterine effect on the developing fetus. There is evidence, however, for a matroclinous effect on the size of the fetal gonads, at least in males, in C57BL and BALB mice (Argyropoulos & Shire, 1989). The two parental contributions could, however, be differentially imprinted. Such a process of imprinting parts of the genome with their parental origins has been shown to underlie both differential inactivation of the *X* chromosome in extra-embryonic tissues (West *et al.* 1977; Monk *et al.* 1987) and also persistent differences in the methylation status of some transgenes (Reik *et al.* 1987; Sapienza *et al.* 1987; Swain *et al.* 1987). In several cases the paternally transmitted information appears to be transmitted in a more active or expressible state than the maternal information (Surani *et al.* 1988).

A model for patroclinous inheritance, which does not assume that methylation is necessarily the cause of inactivation or that the information carrier is necessarily nuclear DNA, is shown in Fig. 2. Two carriers are shown for each mouse because each has two parents, not because the organism has a diploid set of chromosomes. The model requires that the same paternal information state be imposed on all male gametes coming from both testes of an individual. All gametes from an individual are identical, hence there is only one kind (or 'informotype') of offspring produced in any mating, although the transmission potentials for male and females with the same informotype are different. The imprinted state would be expressed in all diploid cells in female offspring (and also in male offspring if the character was not sex-limited to females) but be neutralized or lost during the formation of haploid female gametes. A model based on such assumptions predicts that all the female progeny of an imprinted male will have

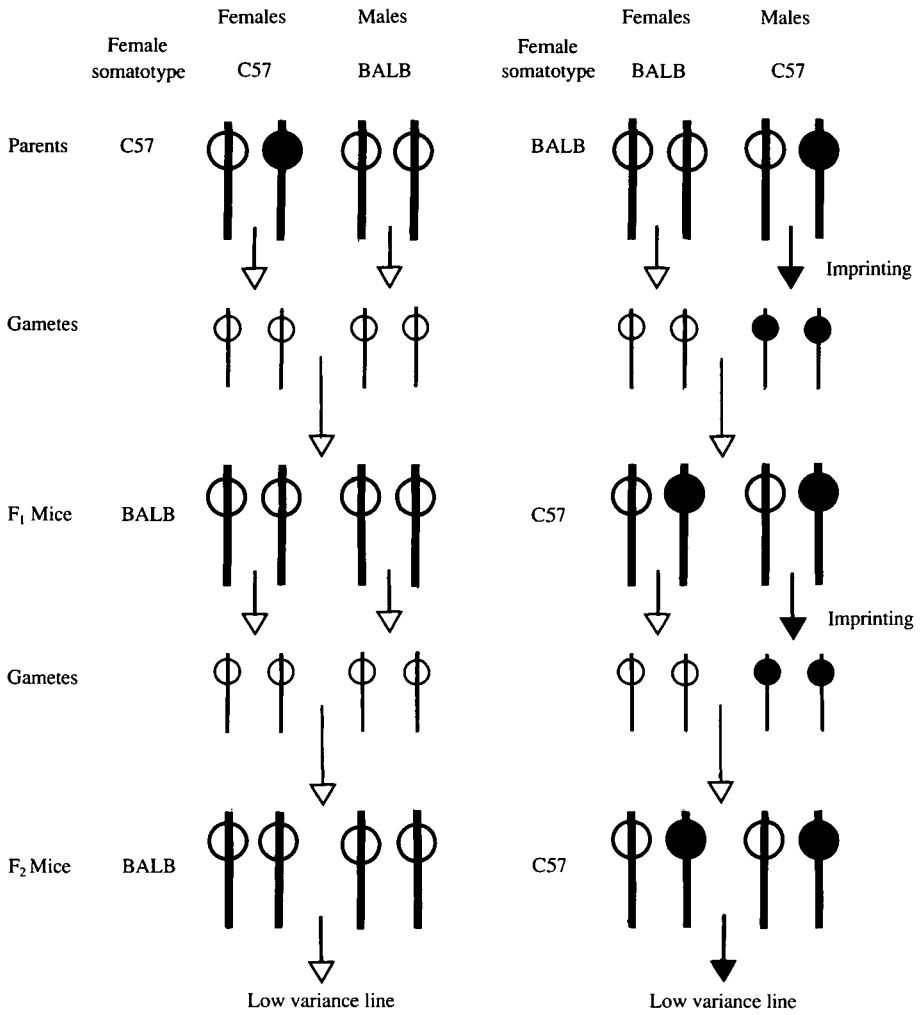

Fig. 2. A model of imprinting mechanisms. The left hand half shows a cross between C57 × BALB, producing B × C F_1 and (B × C) × (B × C) F_2 mice. The right hand half shows the reciprocal BALB × C57 cross, producing C × B F_1 and (C × B) × (C × B) F_2 mice. Each pair of bars represents the information carriers of one mouse. The circles indicate the position of information affecting the trait expressed in the somatic cells of the female mouse of that generation. Filled circles show the positively imprinted state and empty circles the unimprinted or neutral state.

identical phenotypes but will not transmit that trait to their offspring whilst all male progeny will themselves transmit the trait that their sisters express. If an individual male is not imprinted then none of his progeny, either male or female, will show or transmit the imprinted phenotype. Such patterns are shown in the left hand and right hand halves of Fig. 2, and correspond to the pattern of means and variance found for hyaluronidase sensitivity in reciprocal crosses of C57 and BALB. In males imprinting of the altered state of information onto all carrier structures must occur before completion of gametogenesis, but it could occur earlier and might also happen in somatic tissues. The model implies that susceptibility to hyaluronidase was a consequence of an imprinting event in the C57BL paternal lineage, but the data would equally well fit the complementary model in which resistance to hyaluronidase was imprinted in the BALB paternal lineage.

The frequency of occurrence of imprinting in mice may be as high as 15%, based on observations on transgenic variants (Surani *et al.* 1988). Our technique of comparing reciprocal F_1 females for paternally inherited differences is a way of assaying the frequency of systems showing inherited variation in the extent of imprinting, using chromosomally normal mice without any transgenically inserted DNA. Several reports in the literature of patroclinous differences between reciprocal F_1 females suggest that differential imprinting may occur reasonably frequently, and is not restricted to a specific interaction between the C57BL and BALB genotypes. Schabronath & Gartner (1988) have shown that the duration of pronuclear DNA synthesis resembled that of the paternal strain in reciprocal F_1 hybrids between the AKR/NHan and C57BL/6JHan strains. The frequency of diploid oocytes showed a patroclinous pattern in reciprocal F_1 crosses between NMRI mice and both the BALB/c

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and C57BL/6J inbred strains (Beerman *et al.* 1987). Similarly mating DDK females with non-DDK males caused much embryonic lethality yet reciprocal crosses were fully viable (Renard & Babinet, 1986). Interesting the effect was more severe with BALB/c fathers than when they came from the C57BL/6J strain. Patroclinous inheritance of traits affecting tissues other than the germ cells and their immediate investments may also exist. Johnson (1971) found that the volume of embryonic sac fluid in reciprocal F_1 crosses between CBA and C57BL resembled that of their paternal strain. The F_2 mice examined were all from (C57 × CBA) × (C57 × CBA) crosses and were like their CBA forefathers. Some transgenes show maternal hypomethylation (Surani *et al.* 1988), as does the maternal *X* chromosome. Consequently some reciprocal maternal effects in females might thus be due to differences in imprinting. Possible examples are the maternal effect on transient sabling in female $A^y/+$ heterozygotes in the DK strain (Lamoureux & Galbraith, 1986) and reciprocal differences in the thymus growth curves of F_1 hybrids between the NZB and NZW strains (Simpson, 1973). Differences in the severity of effects on offspring of dominant mutations inherited through either the mother or the father are known for both mice (Johnson, 1975; Belyaev *et al.* 1981; Falconer *et al.* 1982) and people (dominant cerebellar ataxia, Harding, 1981; Huntington's chorea, Myers *et al.* 1983; Osteosarcoma, Toguchida *et al.* 1989).

Differences between reciprocal F_1 males are conventionally attributed to *Y*-linked genes if they are like their fathers (Hayward & Shire, 1974; Maxson, 1981; Stewart, 1983; Hunt & Mittwoch, 1987) and to *X*-linked or mitochondrial genes if they resemble their mothers. However, for traits, like testis weight, that are limited in their expression to males *Y*-linked inheritance cannot formally be distinguished from paternal genomic imprinting by conventional breeding tests. The addition of molecular investigations that link phenotypic variation in the trait to a specific DNA sequence on the *Y* chromosome would be required.

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