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Distortion of Mendelian recovery ratio for a mouse HSR is caused by maternal and zygotic effects

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Summary

An HSR in chromosome 1 which is found in many feral populations of *Mus musculus domesticus* was shown in previous studies to consist of a high-copy long-range repeat cluster. One such cluster, *MUT*, showed distorted transmission ratios when introduced by female parents. *MUT*/+ offspring were preferentially recovered at the expense of +/+ embryos in the progeny of ♀ *MUT*/+ × ♂ +/+ but were found at the expected 1:1 ratio in reciprocal crosses. Preferential recovery of maternal *MUT* was due to lethality of postimplantation +/+ embryos. There was no distortion of the recovery ratio in *MUT*/+ × *MUT*/*MUT* progeny: maternal *MUT* and + clusters were present among live implants at a 1:1 ratio. Maternal and zygotic effects therefore contribute to the phenomenon. The mechanism of their interaction is unknown.

1. Introduction

There are various causes of non-Mendelian recovery ratios of alleles in offspring from a heterozygous parent. Biased recovery is due to viability differences between different types of zygotes or gametes (reviews: Lyon, 1991; Lyttle, 1993; Silver, 1993) or to preferential transmission of one allele to more than 50% of gametes at the expense of the other allele ('meiotic drive' *sensu strictu*; review: Ruvinsky, 1995).

Preferential transmission of a chromosome 1 double-band homogeneously staining region (HSR) was found in heterozygous females of *Mus musculus musculus* (Agulnik *et al.*, 1990). The biased recovery of the double-band HSR has been attributed to preferential segregation of the HSR chromosome to the oocyte rather than the polar bodies in female meiosis.

Standard chromosomes 1 harbour a low-copy cluster of long-range repeats (LRRs; ~ 50 copies, repeat length ~ 100 kb, locus *D1Lub1*; Purmann *et al.*, 1992; Traut *et al.*, 1992). Chromosome 1 HSRs are high-copy LRR clusters (⩾ 300 copies; Kunze *et al.*, 1996). They occur as single-band HSRs in *M. m. domesticus* and as double-band HSRs in *M. m. musculus*. The double-band HSR was derived from a single-band HSR by a paracentric inversion during evolution of the semispecies (Winking *et al.*, 1991).

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In the present study, transmission of a *M. m. domesticus* HSR was examined. We show that segregation distortion of the HSR results from post-implantation loss of embryos due to maternal and zygotic effects.

2. Materials and methods

Mouse strains AKR, C57BL/6 (abbreviated B6 in the following) and NMRI contain chromosomes 1 with a low-copy LRR cluster (Kunze *et al.*, 1996 and unpublished data), designated wild-type (+) cluster. The high-copy LRR cluster *MUT* originated from a feral *M. m. domesticus* mouse trapped near Mutten, Switzerland. *MUT* consists of about 900 LRRs (Kunze *et al.*, 1996).

To facilitate identification of chromosome 1, a strain homozygous for the Robertsonian *Rb(1·18)10Rma* chromosome (abbreviated *Rb* in the following) in an NMRI background was used in some crosses. *Rb* harbours the + cluster and, thus, the strain was *Rb*+/*Rb*+. We created a new strain by introducing *MUT* into the *Rb* strain. After six backcross generations, homozygous *Rb MUT*/*Rb MUT* mice were generated by interbreeding and maintained as a stock.

Rb homozygous animals do not show enhanced levels of non-disjunction. Non-disjunction is enhanced, however, in *Rb* heterozygous animals (Gropp

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& Winking, 1981). In crosses with these animals, only euploid near-term foetuses were counted.

Preimplantation loss was determined by comparing either the number of blastocytes (day 4 *p.c.*; plug day = 1 *p.c.*) or the number of implantation sites with the respective number of corpora lutea. Postimplantation loss was determined by comparing the number of live embryos (day 10 to day 13 *p.c.*) with that of implantation sites. Genotypes of live embryos (day 10 to day 13 *p.c.*) and live near-term foetuses (day 18 to day 19 *p.c.*) were determined by C-banding of metaphase chromosomes (Sumner, 1972), exploiting the C-band positive staining of *MUT* and the C-band negative staining of the + cluster. (Traut *et al.*, 1984; Kunze *et al.*, 1996). For typing, blastocysts were transferred to slides in a 1% sodium citrate solution and fixed with methanol–acetic acid (3:1, v:v). We performed fluorescence *in situ* hybridization (FISH) with a digoxigenin-labelled probe, MmHSR1015-3 (Weichenhan *et al.*, 1995), detecting the signal with a fluorescein-conjugated antibody (Boehringer, Mannheim) and counterstaining in propidium iodide as described in Traut *et al.* (1992). The *MUT* and the + cluster were distinguished from each other in interphase nuclei by the signal intensity and the size of the signal area. The two signals were different because of the considerable differences in target LRR copy numbers. To test the reliability of our FISH typing, 36 randomly chosen *MUT*/+ or +/+ blastocysts from *MUT*/*MUT* × +/+ and +/+ × +/+ crosses were examined by FISH. The genotypes of all 36 blastocysts, previously unknown to the investigator, were scored correctly, indicating that genotyping of blastocysts by FISH is reliable.

3. Results

(i) *Preferential recovery of* MUT *from heterozygous females*

Transmission of the *M. m. domesticus* high-copy LRR cluster *MUT* from heterozygous females and males was studied in reciprocal crosses between a heterozygous *Rb MUT*/++ and a homozygous ++/++ parent. Paternal *MUT* and + clusters from heterozygous males segregated in a 1:1 ratio, in accordance with Mendelian expectation (Table 1, lines 1, 3 and 5). In the reciprocal cross, however, the maternal *MUT* cluster was preferentially recovered among live foetuses, independent of the genetic background (Table 1, lines 2, 4 and 6). The deviation from the 1:1 ratio may be stronger with an AKR than a B6 background ($0{\cdot}01 < P < 0{\cdot}05$, χ^2 test of homogeneity).

The results might have been caused by a biased transmission of the Robertsonian chromosome. Among offspring, *MUT* was found either at the original location in the chromosome 1 arm of the Robertsonian chromosome, or as a consequence of meiotic recombination proximal to the cluster, in the acrocentric homologue. Similar deviations from the 1:1 ratio in favour of *MUT* were observed in the reciprocal *Rb MUT*: ++ and + *MUT*: *Rb* + classes (data not shown). This indicated that the Robertsonian chromosome did not influence the recovery ratio of the maternal clusters among euploid foetuses. In the following parts of this section, all parental animals harbouring *MUT* were homozygous for *Rb* and, thus, imaginable complications caused by *Rb* heterozygosity were avoided. (For simplicity, the genetic notation of *Rb* is no longer given in the text but continued in the titles of tables.)

(ii) *Embryonic viability and* MUT *recovery at different developmental stages*

To determine the onset of deviation from the 1:1 ratio, we examined offspring from ♀ *MUT*/+ × ♂ +/+ crosses at the blastocyst stage (day 4 *p.c.*) and postimplantation stage (days 10 and 13 *p.c.*). Almost all ovulated oocytes, as counted from the number of corpora lutea, resulted in blastocysts (Table 2).

The *MUT* cluster consists of about 900 LRR copies while the wild-type cluster consists of only about 50 copies (Kunze *et al.*, 1996). This difference can be made visible by FISH and was exploited to genotype the blastocysts (Plate 1). The ratio of the two types of blastocysts, *MUT*/+ and +/+, did not deviate significantly from 1:1 (Table 2, line 1). Mean cell

Table 1. *Segregation of LRR clusters among live near-term euploid foetuses from reciprocal Rb MUT/++ × ++/++ crosses*

Line no.	Parental genotypes		*n*	Offspring		Recovery of *MUT* (%)	Deviation from 1:1 (χ^2 test)
	Female	Male		*MUT*/+	+/+		
1	$++^{NMRI}/++^{NMRI}$	$Rb\ MUT/++^{AKR}$	137	74	63	54	0·9
2	$Rb\ MUT/++^{AKR}$	$++^{NMRI}/++^{NMRI}$	74	57	17	77	21·6**
3	$++^{NMRI}/++^{NMRI}$	$Rb\ MUT/++^{NMRI}$	225	119	106	53	0·8
4	$Rb\ MUT/++^{NMRI}$	$++^{NMRI}/++^{NMRI}$	156	108	48	69	23·1**
5	$++^{NMRI}/++^{NMRI}$	$Rb\ MUT/++^{B6}$	126	60	66	48	0·3
6	$Rb\ MUT/++^{B6}$	$++^{NMRI}/++^{NMRI}$	139	87	52	63	8·8*

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

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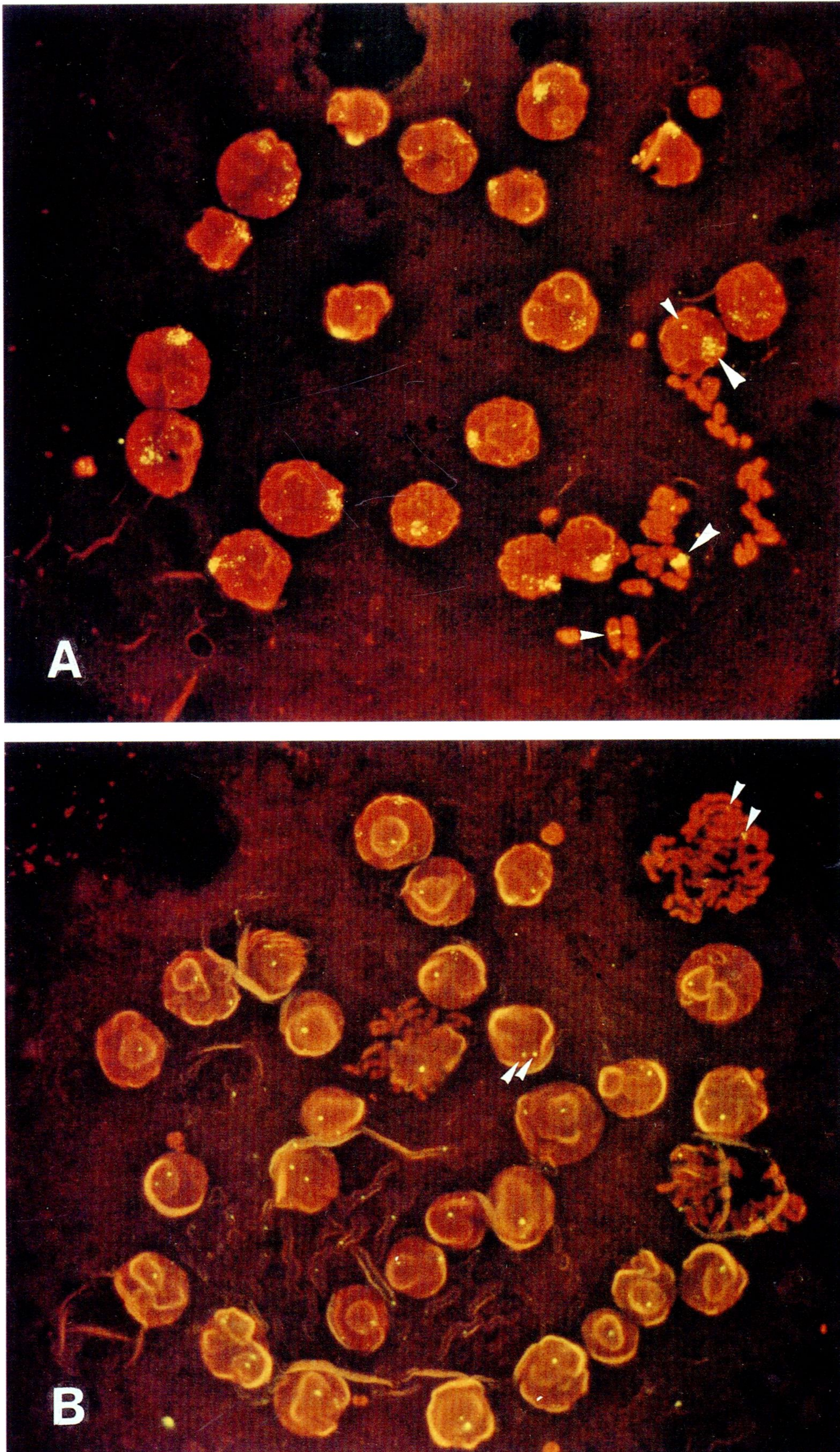

Plate 1. A *MUT*/+ blastocyst (*A*) and a +/+ blastocyst (*B*) genotyped by FISH using an LLR-specific probe. Signal detection was by a fluorescein-labelled antibody, counterstained with propidium iodide. Large arrowheads, *MUT*; small arrowheads, +.

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Table 2. *Blastocysts and postimplantation embryos from ♀ Rb MUT/Rb + × ♂ + +/+ + crosses*

Line no.	Stage of inspection	Female parents	Corpora lutea	Implantation sites	Live embryos *n*	*MUT/+*	+/+	Pre-implantation loss (%)	Post-implantation loss (%)	*MUT/+* (%)	Deviation from 1:1 (χ^2 test)
1	Day 4 *p.c.*	13	175	—	163[a]	70	90	6·9	—	43·8	2·5*
2	Days 10–13 *p.c.*	17	216	203	151	103	48	6·0	25·6	68·2	20·0**

* $0·1 < P < 0·5$; ** $P < 0·001$.
[a] Three recognized triploids were excluded from genotyping.

Table 3. *Postimplantation embryos (days 10–13 p.c.) from ♀ Rb MUT/Rb+ × ♂ + +/+ + crosses and ♀ Rb MUT/Rb+ × ♂ Rb MUT/Rb MUT crosses (females in both types of crosses were derived from the same pool of ♀ Rb MUT/Rb+ × ♂ Rb MUT/Rb+ intercrosses*

Line no.	Parents Female	Male	*n*	Corpora lutea	Implantation sites	Live embryos *n*	*MUT/MUT*	*MUT/+*	+/+	Pre-implantation loss (%)	Post-implantation loss (%)	*MUT/+* (%)	Deviation from 1:1 (χ^2 test)
1	*MUT/+*	+/+	12	152	134	84	—	65	19	11·8	37·3	77·4	25·2*
2	*MUT/+*	*MUT/MUT*	6	88	58	53[a]	23	29	—	34·1	8·6	55·7	0·7

* $P < 0·001$.
[a] One recognized triploid was excluded from genotyping.

numbers per blastocyst showed no significant difference between the two types of blastocysts (35±13 in *MUT/+* and 32±11 in +/+; mean±S.D.). This indicated that they developed at roughly the same cleavage rate up to this stage.

The inspection between days 10 and 13 *p.c.* revealed that almost all embryos had reached the stage of implantation (Table 2, line 2). Between the implantation stage and before the time of inspection, however, a considerable proportion of embryos had been resorbed. Among the live implants, the ratio between *MUT/+* and +/+ had shifted to a preponderance of *MUT/+*. The shift must have been a result of different survival rates of the two classes. This is confirmed when the number of implantation sites is compared with the respective numbers of the two embryo classes. The number of *MUT/+* postimplantation embryos amounted to approximately half, and that of +/+ embryos to a quarter of the number of implantation sites (Table 2, line 2). This indicates near-complete survival of the *MUT/+* class and considerable losses in the +/+ class.

(iii) *Influence of paternal* MUT *on embryonic viability*

MUT/+ females were mated with +/+ males and *MUT/MUT* males to examine the influence of paternal *MUT*. In crosses with +/+ males, postimplantation losses and preferential recovery of *MUT/+* among the embryos (Table 3, line 1) were approximately as observed in the previous experiments. In crosses with *MUT/MUT* males, paternal *MUT* restored the 1:1 ratio of maternal clusters and decreased postimplantation losses (Table 3, line 2). Preimplantation losses, however, were higher than those in the corresponding crosses with +/+ males. We suspect that this is due to reduced sperm fertility of *MUT/MUT* males.

4. Discussion

(i) *Predominant lethality of +/+ postimplantation embryos*

This study describes and analyses the non-Mendelian recovery phenomenon of two LRR clusters: the low-copy wild-type cluster (+) and the high-copy cluster *MUT* from heterozygous *M. m. domesticus* females. Among offspring from *MUT/+* females mated to +/+ males, recovery of *MUT* was significantly higher than that of the maternal + cluster. The reciprocal cross, however, yielded the two classes of offspring in the Mendelian 1:1 ratio. Hence, the non-Mendelian recovery phenomenon is caused by a maternal effect.

At the blastocyst stage (day 4 *p.c.*), a 1:1 ratio between *MUT/+* and +/+ embryos was seen. Among postimplantation embryos (days 10 to 13 *p.c.*), however, the distribution was skewed. Closer investigation revealed high lethality among +/+ embryos between the implantation stage and days 10 to 13 *p.c.* In contrast, *MUT/+* embryos were unaffected. The cause of the biased transmission of maternal clusters,

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therefore, was lethality of a considerable proportion of postimplantation embryos carrying the maternal + cluster. Survivors of the critical period developed normally: there was no appreciable further change in the *MUT*/+ to +/+ ratio between days 10–13 *p.c.* embryos and near-term foetuses.

Distribution of maternal clusters from *MUT*/+ females was not skewed when combined with a paternal *MUT*. We found a 1:1 ratio in postimplantation embryos. Thus, a paternal *MUT* cluster compensates for any adverse effects of the + cluster on progeny from *MUT*/+ females.

The results of Agulnik *et al.* (1990, 1993) in a similar investigation of an LRR cluster are compatible with ours with one notable exception: blastocysts showed the same skewed ratio as live progeny. This pointed to a biased meiotic segregation (meiotic drive *sensu strictu*; Ruvinsky, 1995). In our material, blastocysts presented a normal Mendelian segregation ratio. Due to this difference, we reject meiotic drive as an interpretation of the underlying mechanism in our material.

The difference may lie in the source of animals. Agulnik *et al.* (1990, 1993) investigated transmission of a *M. m. musculus* HSR while we used an HSR from a *M. m. domesticus* population. It is worth noting in this context that high-copy clusters from the two semispecies differ in at least two properties. First, the *domesticus* cluster occurs as one contiguous cluster while the *musculus* cluster is split (Winking *et al.*, 1991). Second, the *domesticus* cluster encodes a family of five, the *musculus* cluster a family of six LRR transcripts, visible in Northern blots (Weichenhan *et al.*, 1995). We cannot be sure, however, whether it is the cluster itself or genes closely linked to it that produce the observed effects.

The methods of genotyping may also have contributed to the different results. Agulnik *et al.* (1990) typed blastocysts from mitotic chromosomes while we used FISH on interphase nuclei and thus were able to genotype all blastocysts.

(ii) *Models for +/+ postimplantation lethality*

The observed interaction of maternal and zygotic factors may take place at different stages of development. We envisage three possible modes of action: genomic imprinting, incompatibility between uterus and embryo, interference with a maternal gene product.

Genomic imprinting is a mechanism which leads to differential expression of genes depending on their passage through the female or male germ line (Solter, 1988). In the *MUT*/+ female germ line, imprinting might affect the + cluster (or a linked gene; for simplicity, this reservation is omitted in the following text) to make it less able to sustain normal development. While paternal *MUT* is able to provide the necessary functions, paternal + is unable to do so. In a search for imprinted chromosome regions, disomies of paternal chromosome 1 have been recovered significantly less frequently than disomies of maternal chromosome 1 (review: Cattanach & Beechey, 1989). No clear-cut imprinting, however, has been discovered yet for chromosome 1 (Beechey & Cattanach, 1995).

Postimplantation lethality might alternatively be caused by incompatibility between the embryo and the uterus. This implies a uterine environment of *MUT*/+ females different from that of +/+ females. In this scenario, +/+ embryos have a reduced prospect of survival in the uterus of *MUT*/+ mothers compared with the uterus of +/+ mothers. Zygotic *MUT*, transmitted either from the mother or from the father, allows the embryo to accommodate to the *MUT*/+ uterine environment, leading to normal viability.

Maternal effects may also be caused by maternal gene products transmitted to the developing embryo via the oocyte or by the lack of such a product. This type of mechanism occurs in *Drosophila* (review: St Johnston & Nüsslein-Volhard, 1992). In the mouse, the 'DDK syndrome' provides a similar case (Renard *et al.*, 1994). In our study, a *MUT*-derived, detrimental cytoplasmic factor, an RNA or a protein, might be the source of the maternal effect in *MUT*-associated postimplantation lethality. If accumulated prior to the second meiotic division in female meiosis, the factor would be incorporated in both types of oocytes from heterozygous *MUT*/+ females. Conceivably, a zygotic *MUT* of maternal or paternal origin inactivates the detrimental factor.

Which of the models really applies to the described phenomena remains to be elucidated. Embryo transfer experiments which are currently under way, such as transfer of +/+ zygotes of *MUT*/+ mothers into +/+ foster mothers, will distinguish between the different hypotheses for the distortion phenomenon among the offspring of *MUT*/+ mothers.

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