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Non-random fertilization in mice correlates with the MHC and something else

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One evolutionary explanation for the success of sexual reproduction assumes that sex is an advantage in the coevolutionary arms race between pathogens and hosts. Accordingly, an important criterion in mate choice and maternal selection thereafter could be the allelic specificity at polymorphic loci involved in parasite–host interactions, e.g. the MHC (major histocompatibility complex). The MHC has been found to influence mate choice and selective abortions in mice and humans. However, it could also influence the fertilization process itself, i.e. (i) the oocyte's choice for the fertilizing sperm, and (ii) the outcome of the second meiotic division after the sperm has entered the egg. We tested both hypotheses in an *in vitro* fertilization experiment with two inbred mouse strains congenic for their MHC. The genotypes of the resulting blastocysts were determined by polymerase chain reaction. We found nonrandom MHC combinations in the blastocysts which may result from both possible choice mechanisms. The outcome changed significantly over time, indicating that a choice for MHC combinations during fertilization may be influenced by one or several external factors.

Keywords: fertilization, gamete choice, MHC, second meiotic division, sexual selection.

Introduction

Some loci which are important in the parasite–host arms race could be under sexual selection as well as under natural selection (Hamilton & Zuk 1982; Wedekind 1994a,b). Accordingly, several studies have shown that the MHC influences mate choice in mice (Yamazaki *et al.*, 1976; Egid & Brown 1989; Potts *et al.*, 1991) and humans (Wedekind *et al.*, 1995) through its effects on body odours and odour perception. Females (or males, respectively) seem to aim for or try to avoid certain alleles according to their own genotype. However, vertebrates are diploid and in nature mostly heterozygous at their MHC loci (Klein, 1986). Therefore, further selection mechanisms after mate choice are necessary to reach an optimal combination of male and female genotypes in the zygote (Wedekind, 1994b).

As one possibility, sperm could somehow signal their haploid specificity at certain loci, and eggs could selectively accept sperms on the basis of these signals. However, there are contradictory reports about whether sperm bear MHC antigens on their cell surface that reveal parts of their haploid genome. On the one hand, gene transcription during spermatogenesis actually seems to occur after the last meiotic division, i.e. from the haploid genome, and protein synthesis continues sometimes afterwards (Eddy *et al.*, 1993). This could explain the finding of some authors that MHC antigens on sperm of mice and humans seem to be haploid expressed (Fellous & Dausset, 1970; Halim & Festenstein, 1975; Arnaiz-Villena & Festenstein, 1976; Halim *et al.*, 1982). On the other hand, however, several groups could not find any MHC antigens on human sperm (Haas & Nahhas, 1986; Kuhlman *et al.*, 1986), and others found MHC antigens on human sperm of only few individuals of a sample (Kurpisz *et al.*, 1987), or found only some HLA-A and -B antigens but not others (Rodríguez-Córdoba & Arnaiz-Villena, 1985). A possible explanation for these controversial findings could be that haploid expression of MHC on sperm is condition-dependent, e.g. depending on the infection status of

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the mouse or human under examination. An infection could be recognized by spermatogenic cells themselves, or probably more likely by cells of tissues outside the testis that are able to communicate with spermatogenic cells. It is known for a number of viruses that they can induce an increased or decreased expression of MHC class I on various body cells (Maudsley *et al.*, 1989; Maudsley & Pound, 1991).

If MHC antigens on sperm can exist at least under certain conditions, then eggs could have receptors for them allowing for a choice of sperm types. These receptors could be located both on the zona pellucida or at the egg membrane itself.

Selection could also occur later, i.e. after the fusion of the gametes during the formation of the second polar body. This is possible because in many vertebrates the second maturational division is completed only after the sperm has penetrated the vitelline membrane of the oocyte (Wolgemuth 1983). The significance of this suspension is not yet clear. It could, however, be an important prerequisite allowing oocytes optimally to complement the sperm's haplotype with their own haplotype at loci like the MHC. However, to make this possible, the oocyte should be able to (i) detect the sperm's haplotype at important loci, and (ii) decide accordingly which of its own haplotypes will stay in the zygote and which will be lost in the second polar body. Furthermore, the oocyte must be still heterozygous for these loci after the first meiotic division. This limits the range of possible choice according to the recombination fraction between these loci and the centromere. For the MHC, this recombination fraction has been estimated to be about 15 per cent (Cui *et al.*, 1992). Therefore, the expected frequency of MHC-heterozygous oocytes during the second meiotic division may be around 28 per cent ($(2 \times 0.15\text{–}0.15^2) \times 100$ per cent).

Several observations indicate that parts of the sperm's haplotype may be 'read' before the oocyte 'decides' which own haplotype will be lost in the second polar body. Shortly after penetration the spermatozoan nuclear envelope disintegrates and the released chromatin material undergoes decondensation for a short time (Wolgemuth, 1983), a process that seems to be controlled by specific components of the oocyte's cytoplasm (e.g. the 'sperm nucleus-decondensing factor') (Wolgemuth, 1983). Processes attending these sperm nuclear transformations and the completion of maternal maturation seem to be tightly linked (e.g. Howlett *et al.*, 1985; Wright & Longo, 1988; Xu & Greve, 1988), and last for about 2 h in mice (Howlett *et al.*, 1985). RNA synthesis has been shown to increase slightly following fertilization of mouse oocytes before fusion of the paternal and maternal pronuclei (Clegg & Pikó, 1982), and protein synthesis after sperm nuclear decondensation is essential for the formation of the male pronucleus (Ding *et al.*, 1992). Moreover, Renard and Babinet (1986) found in an experiment with transplanted pronuclei that some male genes may have been expressed from the decondensed sperm because their products were acting as early as during the one-cell stage. Therefore, it is possible that the outcome of the second meiotic division of the oocyte is influenced by the haplotype of the fertilizing sperm.

In this study we test the following two potential selection mechanisms. (i) Does MHC-dependent choice for the fertilizing sperm occur at the egg wall? (ii) Is the second meiotic division in the egg influenced by the MHC type of the fertilizing sperm?

Materials and methods

We crossed two inbred mouse strains congenic with respect to their *H-2* complex (= the MHC of mice), C57BL/10 ($H\text{-}2^b$) and B10BR ($H\text{-}2^k$), to obtain F1 males and females which were heterozygous at their *H-2* but virtually identical to either parental strain at most other loci. Heterozygous F_1 mice and homozygous C57BL/10 and B10BR mice produced the sperm and oocytes used in the two different *in vitro* fertilization experiments.

First experiment: choice for sperm?

Oocytes of known haplotype (either from a C57BL/10 or B10BR female) were fertilized with sperm from one heterozygous F_1 male, i.e. by a mixture of $H\text{-}2^b$ and $H\text{-}2^k$ bearing sperm from the same male. Figure 1a summarizes the experimental design. To increase the number of oocytes, the females of either parental strain were superovulated by injection i.p. 5 iU PMSG (1. day, 14.00 h) and i.p. 5 iU hCG (3. day, 14.00 h) before removal of the oocytes (4. day, 09.00 h). The *in vitro* fertilization was finished simultaneously for all oocytes at 11.00 h. The oocytes of nine $H\text{-}2^b$ females and of eight $H\text{-}2^k$ females were fertilized *in vitro* with sperm of heterozygous F_1 males. Whenever possible, i.e. in seven cases, the sperm of one male fertilized all eggs from one $H\text{-}2^b$ female and one $H\text{-}2^k$ female. This was to detect an unexpected meiotic drive that would result in an over-representation of one haplotype in the sperm. The formation of the second

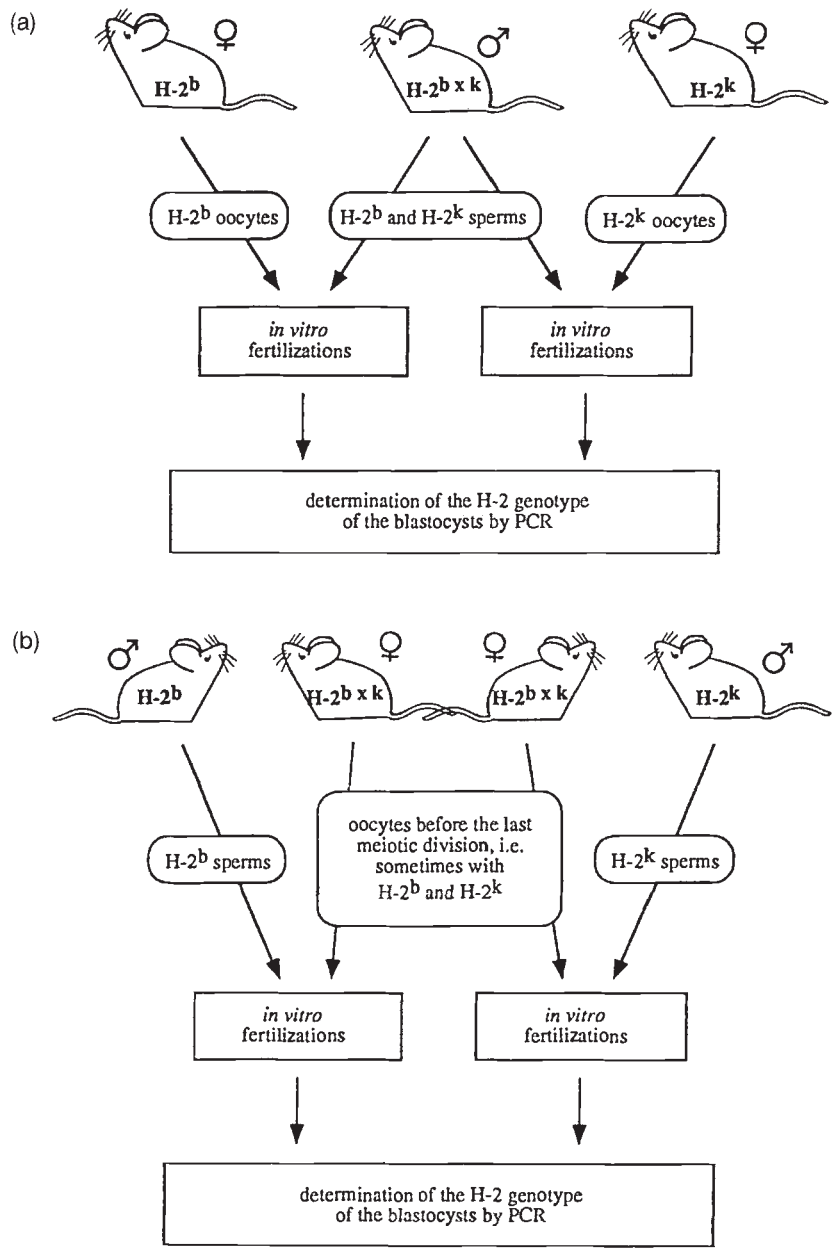

Fig. 1 Schematic overview illustrating the experimental design. Mice of two inbred strains congenic for their MHC and F_1 crosses were used. (a) In the first experiment we tested whether eggs can choose for sperm according to their MHC haplotype (*'H-2'*). (b) In the second experiment, we tested whether the second meiotic division in the egg is influenced by the MHC haplotype fof the fertilizing sperm. See text for details.

polar body and the two pronuclei was checked for every zygote to ensure normal fertilization and to exclude parthenogenetic activation of oocytes (Macas *et al.*, 1993). The zygotes were then cultured (in Ham F 10 medium with 10 per cent human foetal cord serum at 37°C and 5 per cent CO_2 in air) until they reached the blastula stage. We used blastocysts instead of fertilized oocytes for the typing of the MHC genotype to start the amplification of the polymorphic locus with more copies. The blastocysts were transferred to a solution of pronase to remove the zona pellucida and possibe remains of the polar bodies from the embryo. Thereafter, the pretreated blastocysts were transferred into PCR tubes with 5 μL ddH_2O and stored at -70°C. Before the DNA amplification, they were lysed in 10 μL containing 0.05 mg/mL^{-1} proteinase K, 1.7 μM SDS and 20 mM dTT (1 h digestion at 37°C, then 10 min inactivation at 95°C). The *H-2* genotypes of the blastocysts were determined with a polymorphic site at the *Aa* locus,

using the allele-specific amplification associated with allele discrimination by primer length (primers and original method given in Cui *et al.* (1992) see also examples of the detection of *H-2* differences therein). Primers Aa1 and Aa2 were used in a first PCR with the entire blastocyst lysate brought to a final volume of 25 μL (final concentration 10 mM Tris pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 100 μM of each nucleotide, 0.1 μM of each primer, and 0.5 units of Taq polymerase (Perkin) for 40 cycles, with 30 s at 95°C, 2 min at 65°C, and 1 min at 72°C). A second, allele-specific PCR was carried out with primers Aa1, Aa3 and Aa4 on 1 μL aliquot of the first round brought to a final volume of 25 μL (final concentration 10 mM Tris pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 24 μM of each dNTPs (4 μM of them coming from the first PCR), 2 μM of primer Aa1, 2 μM of primer Aa3, between 0.1 and 0.5 μM of primer Aa4, and 0.5 units of Taq polymerase for 25 cycles, with 20 s at 95°C, and 30 s at 58°C). Twenty microlitres of the PCR reactions were analysed by electrophoresis through 8 per cent polyacrylamide gels followed by ethidium bromide staining. As controls, we used blastocysts of crosses within the parental strains (i.e. homozygous at *H-2*, $n = 15$, two of them could not be typed, one homozygous $H\text{-}2^b$ control blastocyst was typed as $H\text{-}2^k$) and between them (i.e. heterozygous at *H-2*, $n = 10$, all were typed correctly).

Second experiment: meiotic division influenced by the haplotype of the fertilizing sperm?

In this experiment, oocytes from *H-2* heterozygous F_1 females were fertilized with sperm of homozygous males, i.e. with sperm of known MHC haplotype. The F_1 females were superovulated as described in the first experiment. The oocytes of 10 F_1 females were fertilized *in vitro* with sperm of $H\text{-}2^b$ males and the oocytes of another ten F_1 females with sperm of $H\text{-}2^k$ males (see Fig. 1b for an illustration of the experimental design). Here again, the formation of the second polar body and the two pronuclei was checked for every zygote to ensure normal fertilization. The culturing, preparation and typing of the embryos were carried out as in the first experiment.

The typing procedure was repeated after all blastocysts (of both experiments) had been typed once (repeating from the second PCR) to gain confidence in the results and to exclude systematic typing errors correlated with the time-course of the experiments.

The data analysis was performed with SYSTAT (1992, version for Macintosh-computer).

Results

In vitro *fertilization and typing of the blastocysts*

In the first experiment, we obtained an average of 6.9 mature oocytes per $H\text{-}2^b$ female and 9.7 mature oocytes per $H\text{-}2^k$ female (including two females from which we obtained no oocytes at all) (Mann–Whitney *U*-test, $U = 35.5$, $P = 0.27$, two-tailed). These results of superovulation are satisfying if we take into account that we used adult females (8 ± 1 weeks), that inbred strains have generally a bad response to the hormone treatment, and that the animals were kept under conventional conditions. The fertilization rate was about the same in both strains (95.7 per cent for $H\text{-}2^b$ eggs and 97.9 per cent for $H\text{-}2^k$ eggs). The survival rate until blastula stage was 71.2 per cent in $H\text{-}2^b$ females and 78.9 per cent in $H\text{-}2^k$ females ($G_1 = 1.26$, $P > 0.20$). We obtained 121 blastocysts, 13 of which could not be typed. The maternal source played a role here, because nine of the 46 blastocysts from $H\text{-}2^b$ females could not be typed, whereas only four of 75 blastocysts from $H\text{-}2^k$ females could not be typed (Fisher's exact test, $P = 0.03$, two-tailed). In some typing, only the paternal haplotype was detected but not the known maternal haplotype. This probability was again different for both haplotypes: four of 15 maternal $H\text{-}2^b$ bands were missing, whereas only one of 36 maternal $H\text{-}2^k$ bands were not detectable in those blastocysts that were typed as heterozygous (Fisher's exact test, $P = 0.02$, two-tailed). All these differences may indicate a viability difference between the early embryos of the two types of females.

In the second experiment, we obtained an average of 9.3 mature oocytes per female. The fertilization rate was 96.8 per cent, i.e. comparable to the fertilization rates of the oocytes of homozygous females. However, the survival rate until blastula stage (93.6 per cent) was higher in embryos from heterozygous mothers than in those from homozygous ones ($G_1 = 21.3$, $P < 0.0001$). Fourteen of the 162 blastocysts could not be typed. In contrast to the respective observation in the first experiment, the *H-2* type of the fathering male did not seem to play a role here: nine of the blastocysts that could not be typed were fathered by $H\text{-}2^k$ males (11.1 per cent) and five by $H\text{-}2^b$ males (6.2 per cent) (Fisher's exact test, $P = 0.40$, two-tailed). The expected paternal haplotype was not detected in only one case in which only one expected $H\text{-}2^b$ band was missing.

The data shown in the following analyses and graphs were not corrected for these typing errors for two reasons. First, it is not clear whether to correct simply haplotype-specific or haplotype-specific *and*

depending on whether the expected haplotype comes from the father or the mother. *H-2*^{b} was detected less often than *H-2*^{k} (combined data for the two experiments: Fisher's exact test, $P = 0.04$, two-tailed), and the frequencies of missing bands tended to be smaller in the second experiment than in the first one (Fisher's exact test, $P = 0.09$, two-tailed). Secondly, as the number of mistypings is small, a correction based on the detected typing errors has only slight effects on the analyses and does not change the conclusions.

Choice for specific MHC-combinations?

Overall, the proportion of homozygous and heterozygous blastocysts did not differ significantly (55.9 per cent homozygosity, $Z = 1.87$, $P = 0.06$, two-tailed). In the first experiment 57 of 108 blastocysts were homozygous (= 53 per cent, $Z = 0.58$, NS; blastocysts from *H-2*^{b} females: 59 per cent, $Z = 1.15$, NS; from *H-2*^{k} females: 49 per cent, $Z = 0.10$, NS), and in the second experiment 86 of 148 blastocysts (= 58 per cent, $Z = 1.97$, $P = 0.05$; blastocysts from *H-2*^{b} males: 59 per cent, $Z = 1.61$, NS; from *H-2*^{k} males: 57 per cent, $Z = 1.18$, NS).

However, to take into account the parental source and the time of fertilization, we based the analysis on *blastocysts per pair of parents* as replicates. Over all, the date of *in vitro* fertilization had a significant influence on the frequency of heterozygous offspring (Fig. 2). The frequency of heterozygosity increased continuously over time during the first experiment (Fig. 2, left side). This correlation could be observed for both strains of females independently (see legend of Fig. 2). In contrast, during the second experiment, which took course immediately after the first one, the frequencies for heterozygosity steadily decreased again (Fig. 2, right side). The correlations were again very similar for both strains of males. This observation is analogous to the first experiment (see legend in Fig. 2). These correlations cannot be explained by differences in the early embryos' survival rates between the time of fertilization and reaching the blastula stage as these survival rates did not correlate with the date of *in vitro* fertilization (survival rates per pair of parents in the first experiment correlated with date of *in vitro* fertilization: Spearman's $r_s = 0.099$, $n = 18$, $P > 0.60$; in the second experiment: $r_s = 0.27$, $n = 20$, $P > 0.20$).

During the 11 weeks where both experiments took place, the average frequency of heterozygosity changed quite continuously, irrespective of which choice mechanism was possible for the oocytes (Fig. 3). This suggests that choice for sperm haplotypes at the egg wall and choice for maternal haplotype after fertilization could be both used to combine certain alleles on the MHC according to an external factor that continuously changed during the time-course of our experiments.

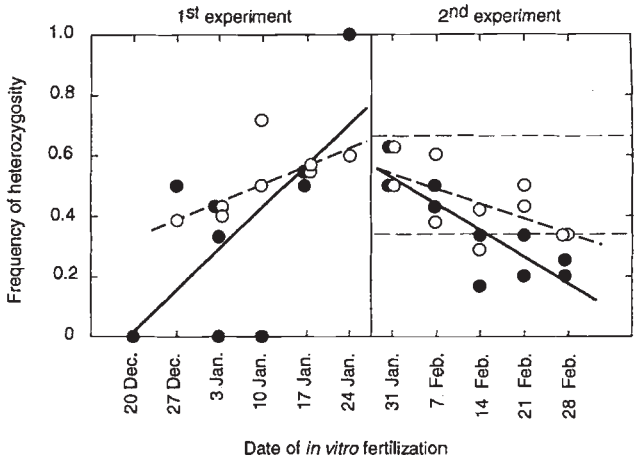

Fig. 2 Frequencies of heterozygous blastocysts per parental pair relative to the date of *in vitro* fertilization. The first experiment took place between 20 December and 24 January, the second from 31 January until 28 February (change indicated by the vertical line). The horizontal dashed lines indicate the expected range of choice in the second experiment depending on the proportion of heterozygous oocytes produced by crossing-over (derived from Cui *et al.*, 1992). The homozygous parent (i.e. the females in the first experiment and the males in the second one) belongs either to the *H-2*^{b} strain (filled circles and solid regression lines) or to the *H-2*^{k} strain (open circles and dashed regression lines). Overall, date had a significant influence on the frequency of heterozygosity per parental pair (Kruskal–Wallis, 22.9, d.f. = 10, $P = 0.01$; without extreme points, i.e. frequencies of 0 and 1: Kruskal–Wallis, 23.3, d.f. = 9, $P = 0.005$). Besides this main analysis, regression lines are drawn solely to describe the pattern of the data: the frequency of heterozygosity increased continuously over time during the first experiment ($r = 0.63$, $n = 17$, $P = 0.006$, two-tailed; without extreme points, i.e. without frequencies of 0 and 1: $r = 0.63$, $n = 13$, $P = 0.02$), both for blastocysts from *H-2*^{b} females ($r = 0.65$) and for blastocysts from *H-2*^{k} females ($r = 0.69$). During the second experiment, the frequencies for heterozygosity continuously decreased ($r = -0.70$, $n = 20$, $P = 0.0006$, two-tailed), for blastocysts fathered by *H-2*^{b} males ($r = -0.84$) as well as for blastocysts fathered by *H-2*^{k} males ($r = -0.63$). These observations suggest that there is choice for certain allele combinations at the MHC both before and after entrance of the fertilizing sperm, and dependent on some external factors correlated with date.

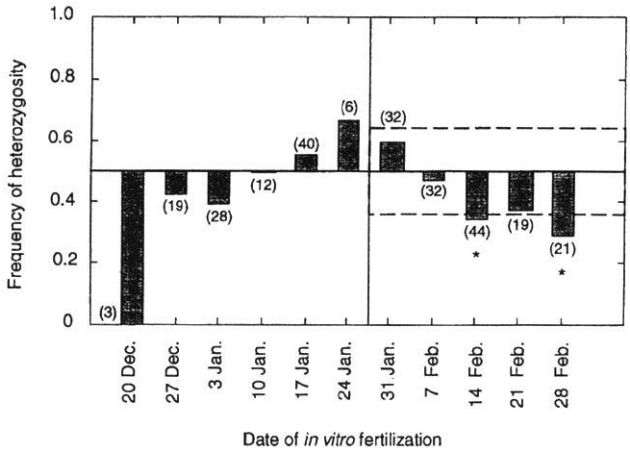

Fig. 3 Deviation from the null-expectancies of the frequencies of heterozygosity per date of *in vitro* fertilization. The vertical line indicates the change from the first to the second experiment, the horizontal dashed lines indicate the expected range of choice that is possible in the second experiment depending on the proportion of heterozygous oocytes produced by crossing-over (derived from Cui *et al.*, 1992). Overall, the signs of the deviations from the null-expectancies change continuously with time (run test for trend data, $n = 11$, $r = 3$, $P = 0.01$, see Sokal & Rohlf, p. 786), indicating that a continuously changing external factor has influenced the choice criteria for the oocyte. The numbers of blastocysts that could be typed per day of *in vitro* fertilization are given in parentheses. (* = Frequency of heterozygosity is significantly different from 0.5 ($Z > 1.96$, $P < 0.05$, two-tailed), but not from 0.36, i.e. from the lower limit of the expected range of possible choice during the second meiotic division ($Z < 0.71$, $P > 0.20$, one-tailed).

Discussion

We have investigated two potential choice mechanisms that could serve to reach specific allele combinations at the MHC, namely whether there is egg choice for different sperm haplotypes and whether the second meiotic division is influenced by the haplotype of the fertilizing sperm.

In both cases, we found nonrandom allelic combinations at the MHC, which changed over the time-course of the experiments. Because of the controlled design, the repetition of the typing procedure after all blastocysts had been typed once, and the fact that we found at times more homozygous and at times more heterozygous blastocysts in both strains, we can exclude several alternative mechanisms that could have explained a deviation from the 50 per cent null expectancy. These include meiotic drive of 'selfish' genomic elements; typing problems associated with better efficiency of the primers for one haplotype; or the observed lower viability of the $H\text{-}2^b$ strain. Unfortunately, in the first experiment we could not avoid a high mortality of the early embryos before the blastula stage. Therefore, we cannot exclude that more heterozygous offspring died at the beginning of this experiment, and more homozygous ones at the end of it, which would produce a pattern such as we observed. However, we would have no explanation for such a differential mortality. In the second experiment, there was only a weak mortality but still a strong time effect on the frequency of heterozygous offspring. This suggests that both choice mechanisms could exist and that they are both MHC *and* condition-dependent, i.e. rely on a not yet identified internal or external factor which changed over time.

It seems to be a general pattern that sexual selection based on the MHC depends on conditional factors. In mice, MHC-dependent mate preferences are not strictly genetically determined but must be acquired during ontogenesis (Yamazaki *et al.*, 1988). In the same species, MHC-dependent spontaneous abortions depend on whether the fathering male is replaced by another male (Yamazaki *et al.*, 1983). Finally, MHC-dependent female preference for male body odours in humans depends on the women's hormonal status, i.e. whether they are using the contraceptive pill (Wedekind *et al.*, 1995).

We do not know which factors did promote the observed changes in allelic combinations over time. One possibility is that the hormonal treatment of the females prior to the removal of the oocytes (to stimulate the superovulations) caused changes in the behaviour of oocytes which may somehow be related to the age of the female donors. However, the timing of the hormonal treatment, of the removal of the oocytes, or of the *in vitro* fertilization is unlikely to have caused an effect here as much effort was taken to keep it equal every week.

Another possibility is that the actual parasite pressure during or before fertilization could have an influence, as MHC-dependent sexual selection has been suggested to be an evolutionary response of hosts to continuously changing parasite pressures (Hamilton & Zuk, 1982; Potts & Wakeland, 1993; Wedekind, 1994a,b).

The mice in our experiment were kept under conventional conditions. Therefore, epidemics, e.g. by mouse hepatitis virus (MHV), frequently occur in the laboratory where the animals for our experiments were kept (T. Rülicke, personal observation). Owing to the immune response of the mice or to medical treatment, these infections usually disappear after a few weeks. Normally, the changes in infection levels are about in the range of time that corre-

sponds with the changes in preference for certain MHC combinations described here. We actually observed a slight epidemic by MHV which peaked at about the middle of our study and which necessitated medical treatment. This epidemic seemed to correlate with the continuous change of the frequency of heterozygosity in the blastocysts. This suggests that an infection during our study might have influenced the outcome of the fertilization. However, testing this explanation will require further experiments.

There is growing evidence that a given allele at the MHC correlates with the host's susceptibility to certain pathogens as well as to certain autoimmune diseases (review in Tiwari & Terasaki, 1985). Therefore, any allele combination may be advantageous under certain environmental conditions but disadvantageous under others, depending on the specificity of parasite pressure. In general, there may be more often a 'better' heterozygous allele combination than would be the homozygous one (e.g. because of codominant expression of the alleles, see Doherty & Zinkernagel, 1975), leading to the observation that in human populations heterozygosity at the MHC is more frequent than expected by chance (Hedrick & Thomson, 1983). However, assuming a general 'heterozygosity advantage' for the MHC might be misleading. Under certain conditions homozygosity of a given allele might be advantageous, e.g. because dose-dependent effects of the gene product. (O'Neill & Blanden (1979) observed that MHC products are sometimes expressed in an up to fourfold lower amount in (heterozygous) F_1 hybrids of two inbred mouse strains compared with the (homozygous) parental lines which were congenic for their MHC. These quantitative differences were also reflected in cytotoxic T cell function.) As a consequence, an animal or an oocyte is expected to prefer a homozygous combination when it can only choose between a 'bad' heterozygous combination and a better homozygous one. Alternatively, the inbred lines in our study could have been artificially selected for traits favouring inbreeding. This could explain the observed overall tendency that they prefer homozygosity over heterozygosity.

Little is known about the selective advantage of specific allele combinations at the MHC. Therefore, it is virtually impossible to predict the outcome of a selection mechanism for optimizing allele combinations under given environmental conditions. Changing environmental conditions could, however, induce changes in the preference.

The two congenic strains are bred to be genetically identical except at the *H-2* region. For this purpose, mice of the strain C57BL/10 have been crossed with C57BL-cd (from where the $H\text{-}2^k$ haplotype derived) to produce the 'congenic' strain B10BR ($H\text{-}2^k$). However, as a consequence of these crossings, the genetic background of the older strain may have been regenerated from some accumulated deleterious mutations. This may explain the viability difference we have found between the two congenic strains: females of the older strain tended to produce fewer mature oocytes, and blastocysts from these females could more often not be typed. The average number of mature oocytes produced by heterozygous females was about in the range of the average number of mature oocytes produced by the $H\text{-}2^k$ females. However, the fertilized oocytes of heterozygous females developed at a much higher rate until blastula stage. This indicates that the early embryos' survival depends on the maternal genotype: offspring of heterozygous F_1 females seem to profit from a general heterozygosity advantage transmitted by cytoplasmic factors of the oocyte.

As embryos of heterozygous females showed a higher survival, early embryos which were heterozygous themselves may be expected to be more viable, too. However, in both experiments we found slightly more homozygous offspring at the blastula stage overall. This suggests that the survival rate of an embryo until the blastula stage does not strongly depend on whether the embryo is homo- or heterozygous.

There is still a debate about whether sperm show haploid expression of the MHC (see Introduction). Our data suggest that sperm differ according to their haplotype, and that this difference can be recognized by oocytes. This could be because sperm express, at least under certain conditions, MHC antigens on their cell surface in a haploid manner (see Introduction). Alternatively, they could differ because of genes located near their MHC. The *H-2* complex spans some 2000 kb DNA and contains nearly 100 genes, many of them encoding products whose function is still unknown (Klein, 1986). Therefore, the observed differences between the congenic strains could also be caused by genes not involved in immune response.

MHC-dependent sexual selection could act on many different levels between mate choice and weaning of the litter (Wedekind 1994b), and for some of these levels evidence already exists that this type of selection actually takes place (e.g. MHC-dependent mate choice: Yamazaki *et al.*, 1976, Egid & Brown, 1989, Potts *et al.*, 1991, Wedekind *et al.*, 1995; MHC-dependent abortions: Yamazaki *et al.*, 1983; Beer *et al.*, 1985; Bolis *et al.*, 1985; Thomas *et*

al., 1985; Karl *et al.*, 1989; Ho *et al.*, 1990; Koyama *et al.*, 1991; Reznikoff Etievant *et al.*, 1991; Laitinen, 1993). The unexpected changes over time in allelic combinations at the MHC found in our experiments suggest that choice mechanisms during fertilization might occur, too. However, little is known about the possible regulatory mechanisms that allow for condition-dependent selection at all these different stages.

Blalock (1984, 1994) suggested that the immune system might act as a sensory system 'feeling' for pathogens and communicating with the nervous system back and forth. As in both choice mechanisms we have investigated it is most probably the oocyte that decides, and a female may 'programme' her oocytes prior to ovulation to make their choice according to the present pathogens. Alternatively, sperm could, together with MHC glycoproteins on their membrane, directly present peptides stemming from a pathogen.

Both selection levels we have investigated here have been found to be operating by other authors. Gametes of many lower organisms (yeast, algae, fungi and protozoa) seem to be able to choose their partner for fusion (Pagel, 1993 and references therein). Moreover, egg choice for sperm has been observed in *Botryllus* sp., a colonial tunicate (Scofield *et al.*, 1982): *Botryllus* eggs resisted fertilization by sperm with the same allele on the fusibility locus for a longer time than sperm with a strange allele on it. A nonrandom completion of the second meiotic division which is influenced by the genotype of the fertilizing sperm has been found in wild populations of Siberian mice (Agulnik *et al.*, 1993); they sometimes have an aberrant chromosome 1 with a large fragment of amplified DNA (called '*ln*'). If the fertilizing sperm is wild-type, then the maternal *ln* chromatid has a much higher chance of going into the zygote than the maternal wild-type chromatid. However, the segregation is normalized if the fertilizing sperm carries *ln* itself, leading to an 1:1 segregation. It still remains unclear whether *ln*/+ mice have a selective advantage over +/+ mice, i.e. whether this meiotic drive increases the zygote's fitness or is just a 'curious side-effect of a curious gene' (Pomiankowski & Hurst, 1993).

In the case of the MHC, however, choosing for optimal allele combinations during fertilization could be adaptive, as it allows for a rapid response to the very important selection pressure by infectious diseases. One of the most substantial benefits of sexual reproduction itself could be that it allows to react to the continuously changing selection pressure induced by infectious diseases (Hamilton *et al.*, 1990, Howard & Lively, 1994). This counteraction would be most efficient with well-tuned condition-dependent selection mechanisms during mate choice, fertilization and early pregnancy.

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