The Royal Society

MHC-genotype of progeny influenced by parental infection

Thomas Rülicke[1], Michel Chapuisat[2]†, Felix R. Homberger[3]‡, Ervin Macas[4] and Claus Wedekind[5]*

[1]*Biologisches Zentrallabor, Universitätsspital Zürich, 8091 Zürich, Switzerland*
[2]*Musée de Zoologie, CP 448, 1017 Lausanne; and Institut de Zoologie et d'Ecologie Animale, Bâtiment de Biologie, Université de Lausanne, 1015 Lausanne, Switzerland*
[3]*Institut für Labortierkunde, Universität Zürich, 8057 Zürich, Switzerland*
[4]*Departement für Frauenheilkunde, Klink für Endokrinologie, Universitätsspital Zürich, 8091 Zürich, Switzerland*
[5]*Abteilung Verhaltensökologie, Zoologisches Institut, Universität Bern, 3032 Hinterkappelen, Switzerland*

In a previous series of *in vitro* fertilization experiments with mice we found non-random combination of major histocompatibility complex (MHC) haplotypes in the very early embryos. Our results suggested that two selection mechanisms were operating: (i) the eggs selected specific sperm; and (ii) the second meiotic division in the eggs was influenced by the type of sperm that entered the egg. Furthermore, the proportion of MHC-heterozygous embryos varied over time, suggesting that non-random fertilization was dependent on an external factor that changed over time. As a higher frequency of heterozygous individuals correlated with an uncontrolled epidemic by MHV (mouse hepatitis virus), we suggested that MHV-infection might have influenced the outcome of fertilization. Here, we present an experiment that tests this hypothesis. We infected randomly chosen mice with MHV and sham-infected control mice five days before pairing. We recovered the two-cell embryos from the oviduct, cultured them until the blastocyst stage, and determined the genotype of each resulting blastocyst by polymerase chain reaction. We found the pattern that we expected from our previous experiments: virus-infected mice produced more MHC-heterozygous embryos than sham-infected ones. This suggests that parents are able to promote specific combinations of MHC-haplotypes during fertilization according to the presence or absence of a viral infection.

Keywords: MHC; sexual selection; mice; mouse hepatitis virus; fertilization; female choice

1. INTRODUCTION

The MHC (major histocompatibility complex) is a group of highly polymorphic genes known to be crucial in parasite–host interaction (Klein 1986). The MHC has therefore been thought to be, either, under natural selection by some sort of parasite-driven balancing selection, or under sexual selection (see recent reviews in Potts & Wakeland 1993; Brown & Eklund 1994; Hedrick 1994; Apanius *et al.* 1997). MHC-correlated mate preferences have been found in several mammalian species such as mice (Yamazaki *et al.* 1976, 1979, 1983, 1994; Egid & Brown 1989; Potts *et al.* 1991), rats (Singh *et al.* 1987; Brown *et al.* 1989), and humans (Wedekind *et al.* 1995; Wedekind & Füri 1997; Ober *et al.* 1997, but see Hedrick & Black 1997). In these examples the MHC appears to bias mate choice through its effects on body odours and odour perception.

Sexual selection is not restricted to mate choice. It includes cryptic selection within the female reproductive tract (Eberhard 1996). Such maternal selection can potentially occur at seven different levels (Wedekind 1994). Yamazaki *et al.* (1983) demonstrated experimentally that pregnancy termination in mice can be induced by MHC-correlated odours, and we found that the fertilization process itself is not random with respect to the MHC of the gametes (Wedekind *et al.* 1996). Our finding was based on a series of *in vitro* fertilization experiments with two inbred mouse strains congenic for their MHC. The observed non-random combinations of MHC haplotypes were the result of selection at two separate levels: (i) the oocyte's selective acceptance of sperm; and (ii) selective second meiotic division after the sperm has entered the egg. However, in our experiments it also became evident that MHC-correlated selection is influenced conditionally by at least one extrinsic factor. We hypothesized that an uncontrolled epidemic by MHV (mouse hepatitis virus) during the course of our experiments had caused a change in the outcome of this selection. The epidemic appeared to enhance the frequency of heterozygosity in the embryos, whereas an epidemic-free situation seemed to lead to more homozygous offspring. Here we present an experiment that tests this hypothesis.

2. METHODS

The experimental design is summarized in figure 1. We crossed two inbred mouse strains congenic with respect to their MHC,

*Author for correspondence (wedekind@esh.unibe.ch).
†Present address: School of Genetics, La Trobe University, Bundoora, 3083 Victoria, Australia.
‡Present address: Section of Comparative Medicine, Yale University School of Medicine, PO Box 208016, New Haven, CT 06520-8016, USA.

Received 4 December 1997 *Accepted* 19 January 1998

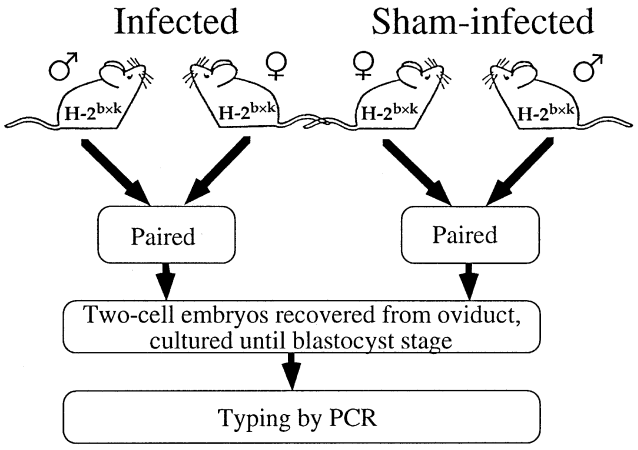

Figure 1. Schematic illustration of the experimental design. The mice used were F1 crosses of two inbred mice congenic for their MHC, i.e. they were heterozygous on the MHC and homozygous on most if not all other loci. See text for details.

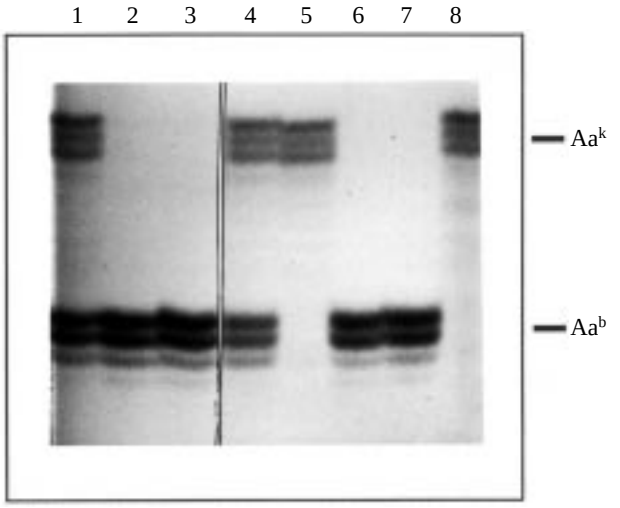

Figure 2. MHC-genotypes of mice blastocysts. Blastocysts from sham-infected parents (1–3), blastocysts from MHV-infected parents (4–6), control blastocyst H-2^b (7), control blastocyst H-2^k (8).

C57BL–10 (H-2^b) and Bl0BR (H-2^k), to get F1 progeny which were heterozygous at their MHC but homozygous at most other loci. All mice were kept under specified pathogen-free conditions (FELASA recommendations, see Rehbinder *et al.* 1996). When seven- to nine-weeks-old, F1 mice of both sexes were either infected *per oral* with 10 µl of an enterotropic wild-type strain of MHV ('infected') or treated with 10 µl physiological NaCl-solution *per oral* ('sham-infected'), respectively (the inoculum consisted of 10% infant mouse intestinal homogenate with 10^6–10^7 ID_{50}; for the origin of the MHV sub-strain, see Homberger *et al.* (1998)). The females were then treated for super ovulation to increase the number of oocytes by intraperitoneal injection of 5 iU PMSG (3 d post-infection) and 5 iU hCG intraperitoneally 48 h later (distributor of PMSG: Folligon®, Intervet; of hCG: Pregnyl®, Organon). On that day (respectively 5 d post-infection) males and females were paired within each experimental group (15 pairs per group). Thus, fertilization occurred at the time of highest virus titres (Barthold *et al.* 1993). After 42 h post-hCG-injection, we recovered the two-cell embryos from the oviduct. In six of the sham-infected and in one of the infected females we could not recover any living two-cell embryos (but often many degenerated or unfertilized eggs). These females were excluded from all further analyses.

We cultured the recovered two-cell embryos for 76 h (until they reached the blastocyst stage) in modified Biggers' medium (Biggers *et al.* 1971) at 37 °C and 5% CO_2 in air. The blastocysts were then transferred to a solution of pronase E (Sigma) to remove the zona pellucida and, possibly, existing remains of the polar bodies. They were then transferred singly into PCR tubes with 5 µl of double-distilled H_2O and stored at −70 °C until all the blastocysts from each pairing had been collected. The H-2 genotype of each blastocyst was determined with allele-specific PCR based on a polymorphic site at the *Aa* locus, and associated with allele discrimination by primer length (Cui *et al.* 1992): in a second, nested PCR, alleles were amplified with specific primers differing in their 3′-end, and were discriminated on the basis of the length of the amplification product, because the Aa^k-specific primer had a 10-bp tail attached to its 5′-end (Cui *et al.* 1992). We used the primers given in Cui *et al.* (1992) and followed the methods detailed in Wedekind *et al.* (1996), with the following modifications which clearly increased typing efficiency. The second, allele-specific, PCR was done in a final volume of 10 µl with 2 µM of each dNTP and 0.02 µl of 33P-dATP. The amplification products were separated on sequencing gels (6% acrylamide, 8 M urea) and visualized by autoradiography (see figure 2). We used blastocysts instead of two-cell embryos to minimize errors associated with amplification of low copy number genes. A total of 16 blastocysts of known genotype served as controls. If a band appeared these controls were always correctly typed (three could not be typed).

To test whether the infection procedure was successful we collected ascending colon from both parents for a virus assay by PCR (Homberger *et al.* 1991). This assay was negative for all sham-infected mice and positive for all MHV-infected ones except for two males.

3. RESULTS

On average, 15.9% of the embryos per female were degenerated and therefore could not be used for typing. The number of degenerated embryos was not different in the two experimental groups ($t=-0.20$, $p=0.84$). We obtained an average of 16.6 (s.d.=6.0) blastocysts per female of which, on average, 15.5 (s.d.=5.5) could be typed for their MHC (the efficiency of typing was not significantly different between the two experimental groups, $t=0.59$, $p=0.56$; one female from which we obtained only two blastocysts was omitted from the further analysis). Overall, the average number of embryos that were degenerated or could not be typed for other reasons were 4.1 (s.d.=3.4) and 4.2 (s.d.=1.9) for infected parents and sham-infected parents, respectively ($t=0.06$, $p=0.95$).

MHV-infected parents produced significantly more MHC-heterozygous embryos than sham-infected ones (see figure 3). The analysis in figure 3 was based on frequencies of MHC heterozygotes per pair of parents, to take parental effects into account (and to avoid pseudo-replication, see Hurlbert (1984)). However, in both groups the result was not significantly different from 50% heterozygosity (infected females, $t=1.41$, $p=0.18$;

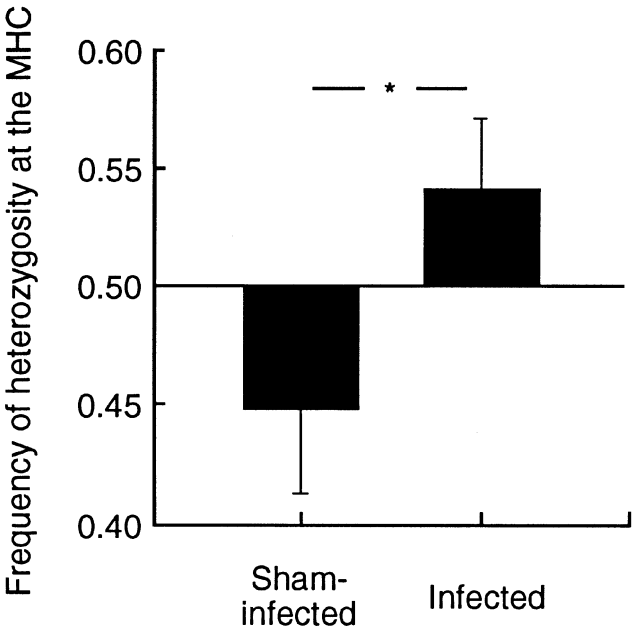

Figure 3. Frequency of MHC-heterozygotes per pair of parents who were either infected with mouse hepatitis virus or sham-infected. These frequencies were different for infected and sham-infected pairs of parents ($t=2.04$, $p=0.03$, directed (Rice & Gaines 1994)) according to the prediction of our previous study (Wedekind *et al*. 1996; when tested two-tailed the p-value would be 0.05).

sham-infected females, $t=-1.49$, $p=0.18$, two-tailed). The frequencies of the two haplotypes in the embryos were also not significantly different between sham-infected and infected mice (average frequency of the H-2^k haplotype in sham-infected females, 0.467, s.e.=0.02; in infected females, 0.538, s.e.=0.03; comparison: $t=-1.77$, $p=0.09$, two-tailed).

4. DISCUSSION

Virus-infected mice produced more MHC-heterozygous embryos than non-infected ones. This suggests that parents are able to promote the specific combination of MHC-haplotypes according to the presence or absence of a viral infection. Parents could achieve this after intercellular signalling events by egg selection for sperm and/or by selectively biasing the outcome of the second meiotic division after the sperm has entered the egg. Our previous experiments suggest that both selection levels affect the MHC-combination of the embryos (Wedekind *et al.* 1996). MHC-dependent selection by the virus itself on the very early embryos is unlikely to have had an influence on our findings. Preimplantation embryos are protected against infections by MHV and resulting cytopathic effects as long as the zona pellucida is intact (Reetz *et al.* 1988). Furthermore, the proportion of blastocysts that could not be typed did not differ significantly between the two experimental groups.

Several authors have previously searched for deviations from the expected ratios of MHC-heterozygosity in the progeny of controlled-matings. They reported a significant variability of MHC-heterozygote frequencies which, however, remained poorly understood because there appeared to be a general inability to replicate previous findings (see discussion in Hings & Billingham (1985)). The outcome of our experiment could lead to an explanation for the controversial findings published before, as we could do our experiments under defined hygienic conditions with a selective and monitored viral infection, i.e. we could control for a factor (infection) that was not controlled in the previous studies and that could have influenced their outcome.

The list of papers starts with Gorer & Mikulska (1959). They reported significant deviations from the expected ratios of MHC-heterozygous progeny of backcross matings in mice. However, the excess of MHC-heterozygotes was reduced in later phases of their breeding programme. Gorer & Mikulska speculated that a parasite temporarily existing in the colony might have caused this change over time by having a more pronounced adverse effect on MHC-homozygous offspring. Palm (1969) performed controlled pairings of rats to test whether she could find more MHC-heterozygotes at weaning than expected by chance. In a first set of experiments with seven types of crossings and 466 offspring, she found a significant excess of heterozygotes (55.8% compared with the 50% null-expectancy). In a second set of experiments with four types of crossings and 402 offspring she found only a slight, and overall not significant, excess of heterozygotes (53.0%). However, the data of this second set were very heterogeneous: in some matings an excess of heterozygotes was obvious, in others no such excess was visible. Overall, the maternal genotype seemed to influence the distribution of MHC-alleles in the progeny, but not in a simple deterministic way. Palm (1969) mentioned a very high preweaning loss of progeny in her experiments, suggesting that either a non-identified selective agent (e.g. a pathogen) could have produced the results through selectively favouring heterozygotes (natural selection), or that such a selective agent could have influenced a possible maternal selection for certain MHC-genotypes (sexual selection). Palm then conducted a third set of experiments (Palm 1970) in which she observed no overall significant excess of MHC-heterozygotes or MHC-homozygotes. The unexplained mortality in this third set was about five times smaller than in the previous experiments.

Hings & Billingham (1981, 1983, 1985) attempted to reproduce Palm's findings. In their first study (Hings & Billingham 1981) they found a significant excess of MHC-heterozygous male offspring in untreated rats but no deviation from Mendelian expectations when parents were treated by an antibiotic (tetracycline in drinking water). As the offspring of untreated rats experienced a much higher mortality rate than the offspring of rats treated with antibiotic, they concluded that the difference may be largely owing to an infection. In their second set of experiments, Hings & Billingham (1983) compared the frequencies of MHC-heterozygous offspring in increasing parity. In all the set-ups the percentage of heterozygotes was between 44.1% and 55.8%, and in most of them, increasing parity did not correlate with the frequency of MHC-heterozygous offspring. However, parents treated with tetracycline showed a significant decrease of MHC-heterozygosity from the first to the last litters (55.7–44.9%), i.e. the longer the rats have been treated with antibiotics, the higher the frequency of MHC-homozygous offspring they produced. This change was associated with

a slight and not significant decrease in mortality. After two years, the same authors presented another large-scale study (Hings & Billingham 1985) where they again found a significant decrease of MHC-heterozygosity from the first to the last litter in parents treated with tetracycline (55.4–42.7%), this time associated with a clear decrease in infant mortality from the first three litters to the last one.

All these experiments did not allow for a discrimination between the two alternative hypotheses, namely whether the excess of heterozygotes was the result of selection by the infection itself or the result of a preference for heterozygous offspring by the infected mothers. However, another interesting finding of the last study by Hings & Billingham (1985) was that a certain strain combination tested was found to result in a significant excess of heterozygous offspring, but only if the strain combination was F1 female × F1–DA male instead of F1–DA female × F1 male. The interesting point here is that the offspring of these two matings are expected to be, on average, of the same MHC-genotypes, i.e. the excess of heterozygosity of the one pairing is unlikely to be simply the result of a general increase in vigour of MHC-heterozygote offspring. Factors other than that appeared to play a role in determining the excess of MHC-heterozygotes.

The next data set on mice was derived by Potts *et al.* (1991). They tested specifically for MHC-related selective-fertilization or abortion by controlled laboratory matings. When analysing the MHC-types of embryos, they found a 6% excess of MHC-heterozygotes which was, however, statistically not significant. The authors suggested that their colonies were loaded with pathogens as they were wild-derived and no special treatment was undertaken to keep the mice uninfected. Hence, these mice may be comparable, to some degree, to the infected mice in our study.

Most deviations from the null-expectancy reported in these previous studies were within the range of deviation reported in the present one, i.e. around ±5%. Furthermore, an excess of MHC-heterozygotes appeared to be more likely when mortality rates among the preweaning offspring were high, i.e. under conditions that indicate an epidemic in the laboratory at the time the experiment took place. Animals treated with tetracycline produced less MHC-heterozygotes the longer they had been treated. At the end of treatment their frequency of MHC-homozygous offspring was in the range of the non-infected animals in the present study.

In some other taxa, non-random fertilization is a well-known phenomenon. In a population of sand lizards (*Lacerta agilis*) where most females mate with more than one male, the male's genetic similarity to the female correlates with the proportion of offspring sired by the male: more dissimilar males sire more offspring, both in the field and in the laboratory (Olsson *et al.* 1996). Olsson and co-workers concluded that the female reproductive tissue actively selects for genetically dissimilar sperm (see also Olsson *et al.* 1997). In the ascidian *Diplosoma listeranum*, a colonial, sessile, marine filter-feeder that disperses sperm into surrounding water, sperm are taken-up and pass up the oviduct to reach the oocyte within the ovary. Autoradiography of labelled sperm revealed that sperm from the same clone are normally stopped in the oviduct whereas sperm from other clones progress to the ovary (Bishop 1996). Gametic self-incompatibility has been intensely studied in the hermaphroditic tunicate *Ciona intestinalis* (see, for example, Rosati & de Santis 1978; de Santis & Pinto 1991). Self-discrimination occurs in the vitelline coat, is established there in late oogenesis, and is controlled by products of overlying follicle cells. Self-sterility in this species is not absolute but appears to depend on still unidentified factors (Rosati & de Santis 1978; de Santis & Pinto 1991). Further examples that suggest non-random fertilization are reviewed in Eberhard (1996) and Zeh & Zeh (1997), but Simmons *et al.* (1996) and Stockley (1997) could not find it in yellow dung flies and in common shrews, respectively. Whereas the loci involved in the reproductive compatibility or incompatibility are not yet known in the above examples, they are known in at least several plants and in a tunicate. Growth of the pollen tube is often affected by the stigma and depends on the combination of male and female alleles on the self-incompatibility locus (see, for example, Franklin-Tong & Franklin 1993). In the tunicate *Botryllus* sp., eggs appeared to resist fertilization by sperm with the same allele on the fusibility locus for a longer period than sperm with a different allele on the fusibility locus (Scofield *et al.* 1982).

The physiology behind the conditional selection we have observed here is far from being clarified (see Wedekind *et al.* 1996). However, we can discuss its possible function: the observed selection may be associated with a fitness advantage of certain MHC-combinations under given environmental conditions, e.g. the presence or absence of a viral infection. Homo- or heterozygosity at the MHC affects T-cell receptor repertoire selection (Vukusic *et al.* 1995) and presentation of MHC-antigens on cell surfaces (O'Neill & Blanden 1979). Both could be responsible for a variable expression of autoimmune disease and a variable expression of immune response to an infection (Doherty & Zinkernagel 1975; O'Neill & Blanden 1979; Apt 1990; Bacon & Witter 1995). In humans, for example, it appears that homozygous individuals have generally lower immune responses to hepatitis B vaccines or infection than heterozygous ones (Alper *et al.* 1989; Pollicino *et al.* 1996; Thurz *et al.* 1997). This suggests that producing heterozygous offspring under the threat of an infection by hepatitis could give a fitness advantage. However, not much is known about a possible advantage of homozygous offspring under hepatitis-free conditions in humans. Furthermore, the comparison between human hepatitis B and MHV may be problematic in some respect. There is still a great need for research on the beneficial or deleterious aspects of various homo- or heterozygous combinations of MHC-alleles under given environmental conditions.

5. CONCLUSION

The findings reported here confirm that gamete fusion in mice is not random with respect to the MHC (Wedekind *et al.* 1996), and that it is influenced by an external factor, i.e. an infection. The incidence of epidemics will therefore have an impact on the outcome of gamete fusion. As infections are likely to change over time and differ between different laboratories, it may not be surprising that some previous findings could not be replicated. We therefore suggest that the controversial findings

published before were influenced by the presence or absence of infections. However, the selective advantage of MHC-homozygosity for uninfected animals and MHC-heterozygosity for infected ones remains to be demonstrated. This advantage could be connected, for example, to T-cell ontogeny, i.e. to potential higher costs of T-cell receptor repertoire selection for MHC-heterozygotes, or to the wider range of pathogen-specific peptides that can be presented by MHC-heterozygotes. The former argument could select for MHC-homozygotes under pathogen-free conditions, whereas the latter may select for MHC-heterozygotes under infectious conditions.

We thank Catherine Roger, Kirsten Rappold and Linong Zhang for technical assistance, and Wayne Potts for help with the literature. We also thank Jonathan C. Howard, Manfred Milinski, Dustin Penn, Wayne Potts, Andreas Ziegler, Rolf M. Zinkernagel, and the anonymous referees for discussion and/or helpful comments on the manuscript.

REFERENCES

Alper, C. A., Kruskall, M. S., Marcus-Bagley, D., Craven, D. E., Katz, A. J., Brink, S. J., Dienstag, J. L., Awdeh, Z. & Yunis, E. J. 1989 Genetic prediction of nonresponse to hepatitis B vaccine. *New Engl. J. Med.* **321**, 708–712.

Apanius, V., Penn, D., Slev, P. R., Ruff, L. R. & Potts, W. K. 1997 The nature of selection on the major histocompatibility complex. *Crit. Rev. Immunol.* **17**, 179–224.

Apt, A. S. 1990 MHC heterozygosity and enhanced disease resistance—an example. *Immunol. Today* **11**, 387.

Bacon, L. D. & Witter, R. L. 1995 Efficacy of Marek's disease vaccines in MHC heterozygous chickens: MHC congenic × inbred line F1 matings. *J. Hered.* **86**, 269–273.

Barthold, S. W., Beck, D. S. & Smith, A. L. 1993 Enterotropic coronavirus (mouse hepatitis virus) in mice: influence of host age and strain on infection and disease. *Lab. Anim. Sci.* **43**, 276–284.

Biggers, J. D., Whitten, W. K. & Whittingham, D. G. 1971 Culture of mouse embryos *in vitro*. In *Methods in mammalian embryology* (ed. J. C. Daniel Jr), pp. 86–116. San Francisco: W. H. Freemann & Co.

Bishop, J. D. D. 1996 Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum*. I. Autoradiographic investigation of sperm movement in the female reproductive tract. *Proc. R. Soc. Lond.* B **263**, 369–376.

Brown, J. L. & Eklund, A. 1994 Kin recognition and the major histocompatibility complex: an integrative review. *Am. Nat.* **143**, 435–461.

Brown, R. E., Roser, B. & Singh, P. B. 1989 Class I and class II regions of the major histocompatability complex both contribute to individual odors in congenic inbred strains of rats. *Behav. Genet.* **19**, 659–674.

Cui, X., Gerwin, J., Navidi, W., Li, H., Kuehn, M. & Arnheim, N. 1992 Gene-centromere linkage mapping by PCR analysis of individual oocytes. *Genomics* **13**, 713–717.

de Santis, R. & Pinto, M. R. 1991 Gamete self-discrimination in Ascidians: a role for the follicle cells. *Molec. Reprod. Devl.* **29**, 47–50.

Doherty, P. C. & Zinkernagel, R. M. 1975 Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature* **256**, 50–52.

Eberhard, W. G. 1996 *Female control: sexual selection by cryptic female choice*. Princeton University Press.

Egid, K. & Brown, J. L. 1989 The major histocompatibility complex and female mating preferences in mice. *Anim. Behav.* **38**, 548–550.

Franklin-Tong, N. V. E. & Franklin, C. F. C. H. 1993 Gametophytic self-incompatibility: contrasting mechanisms for *Nicotiana* and *Papaver*. *Trends Cell Biol.* **3**, 340–345.

Gorer, P. A. & Mikulska, Z. B. 1959 Some further data on the H-2 system of antigens. *Proc. R. Soc. Lond.* B **151**, 57–69.

Hedrick, P. W. 1994 Evolutionary genetics of the major histocompatibility complex. *Am. Nat.* **143**, 945–964.

Hedrick, P. W. & Black, F. L. 1997 HLA and mate selection: no evidence in South Amerindians. *Am. J. Hum. Genet.* **61**, 505–511.

Hings, I. M. & Billingham, R. E. 1981 Splenectomy and sensitization of Fischer female rats favors histoincompatibility of R_2 back-cross progeny. *Transpl. Proc.* **13**, 1253–1255.

Hings, I. M. & Billingham, R. E. 1983 Parity-induced changes in the frequency of RT1 heterozygotes in an R_2 backcross. *Transpl. Proc.* **15**, 900–902.

Hings, I. M. & Billingham, R. E. 1985 Maternal–fetal immune interactions and the maintenance of major histocompatibility complex polymorphism in the rat. *J. Reprod. Immunol.* **7**, 337–350.

Homberger, F. R., Smith, A. L. & Barthold, S. W. 1991 Detection of rodent coronavirus in tissues and cell cultures by using polymerase chain reaction. *J. Clin. Microbiol.* **29**, 2789–2793.

Homberger, F. R., Zhang, L. & Barthold, S. W. 1998 Relative prevalence of enterotopic and polytropic mouse hepatitis virus in enzootically infected mouse colonies. *Lab. Anim. Sci.* **48**, 50–54.

Hurlbert, S. H. 1984 Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* **54**, 187–211.

Klein, J. 1986 *Natural history of the major histocompatibility complex*. New York: Wiley.

Ober, C., Weitkamp, L. R., Cox, N., Dytch, H., Kostyu, D. & Elias, S. 1997 HLA and mate choice in humans. *Am. J. Hum. Genet.* **61**, 497–505.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelström, H. 1996 Sperm selection by females. *Nature* **383**, 585.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. & Tegelström, H. 1997 Sperm choice by females. *Trends Ecol. Evol.* **12**, 445–446.

O'Neill, H. C. & Blanden, R. V. 1979 Quantitative differences in the expression of parentally derived H-2 antigens in F1 hybrid mice affect T-cell responses. *J. Exp. Med.* **149**, 724–731.

Palm, J. 1969 Association of maternal genotype and excess heterozygosity for Ag-B histocompatibility antigens among male rats. *Transpl. Proc.* **1**, 82–84.

Palm, J. 1970 Maternal–fetal interaction and histocompatibility antigens polymorphisms. *Transpl. Proc.* **2**, 162–173.

Pollicino, T., Pernice, F., Campo, S., Mesiti, O., Misefari, V., Pernice, M. & Raimondo, G. 1996 Severe outcome of hepatitis B virus (HBV) infection and lack of HBV e antigen-defective virus emergence in patients homozygous for HLA class I alleles. *J. Gen. Virol.* **77**, 1833–1836.

Potts, W. K. & Wakeland, E. K. 1993 Evolution of MHC genetic diversity: a tale of incest, pestilence and sexual preference. *Trends Genet.* **9**, 408–412.

Potts, W. K., Manning, C. J. & Wakeland, E. K. 1991 Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature* **352**, 619–621.

Reetz, I. J., Wullenweber, M., Kraft, V. & Hedrich, H.-J. 1988 Rederivation of inbred strains of mice by means of embryo transfer. *Lab. Anim. Sci.* **38**, 696–701.

Rehbinder, C., Baneux, P., Forbes, D., Van Herck, H., Niklas, W., Rugaya, Z. & Winkler, G. 1996 FELASA recommendations for the health monitoring of mouse, rat, hamster, gerbil, guinea-pig and rabbit experimental units. *Lab. Anim.* **30**, 193–208.

Rice, W. R. & Gaines, S. D. 1994 'Heads I win, tail you lose': testing directional alternative hypotheses in ecological and evolutionary research. *Trends Ecol. Evol.* **9**, 235–237.

Rosati, F. & de Santis, R. 1978 Studies on fertilization in the ascidans. I. Self-sterility and specific recognition between gametes of *Ciona intestinalis*. *Exp. Cell Res.* **112**, 111–119.

Scofield, V. L., Schlumpberger, J. M, West, L. A. & Weissman, I. L. 1982 Protochordate allorecognition is controlled by a MHC-like gene system. *Nature* **295**, 499–502.

Simmons, L. W., Stockley, P., Jackson, R. L. & Parker, G. A. 1996 Sperm competition or sperm selection: no evidence for female influence over paternity in yellow dung flies *Scatophaga stercoraria*. *Behav. Ecol. Sociobiol.* **38**, 199–206.

Singh, P. B., Brown, R. E. & Roser, B. 1987 MHC antigens in urine as olfactory recognition cues. *Nature* **327**, 161–164.

Stockley, P. 1997 No evidence of sperm selection by female common shrews. *Proc. R. Soc. Lond.* B **264**, 1497–1500.

Thurz, M. R., Thomas, H. C., Greenwood, B. M. & Hill, A. V. S. 1997 Heterozygote advantage for HLA class-II type in hepatitis B virus infection. *Nature Genet.* **17**, 11–12.

Vukusic, B., Poplonski, L., Phillips, L., Pawling, J., Delovitch, T., Hozumi, N. & Wither, J. 1995 Both MHC and background gene heterozygosity alter T cell receptor repertoire selection in an antigen-specific response. *Molec. Immunol.* **32**, 1355–1367.

Wedekind, C. 1994 Mate choice and maternal selection for specific parasite resistances before, during and after fertilization. *Phil. Trans. R. Soc. Lond.* B **346**, 303–311.

Wedekind, C. & Füri, E. 1997 Body odour preference in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proc. R. Soc. Lond.* B **264**, 1471–1479.

Wedekind, C., Seebeck, T., Bettens, F. & Paepke, A. J. 1995 MHC-dependent mate preferences in humans. *Proc. R. Soc. Lond.* B **260**, 245–249.

Wedekind, C., Chapuisat, M., Macas, E. & Rülicke, T. 1996 Non-random fertilization in mice correlates with the MHC and something else. *Heredity* **77**, 400–409.

Yamazaki, K., Boyse, E. A., Miké, V., Thaler, H. T., Mathieson, B. J., Abbott, J., Boyse, J., Zayas, Z. A. & Thomas, L. 1976 Control of mating preference in mice by genes in the major histocompatibility complex. *J. Exp. Med.* **144**, 1324–1335.

Yamazaki, K., Yamaguchi, M., Baranoski, L., Bard, J., Boyse, E. A. & Thomas, L. 1979 Recognition among mice. Evidence from the use of a Y-maze differentially scented by congenic mice of different major histocompatibility types. *J. Exp. Med.* **150**, 755–760.

Yamazaki, K., Beauchamp, G. K., Wysocki, C. J., Bard, J., Thomas, L. & Boyse, E. A. 1983 Recognition of H-2 types in relation to the blocking of pregnancy in mice. *Science* **221**, 186–188.

Yamazaki, K., Beauchamp, G. K., Shen, F.-W., Bard, J. & Boyse, E. A. 1994 Discrimination of odor types determined by the major histocompatibility complex among outbred mice. *Proc. Natn. Acad. Sci. USA* **91**, 3735–3738.

Zeh, J. A. & Zeh, D. W. 1997 The evolution of polyandry. II. Postcopulatory defence against genetic incompatibility. *Proc. R. Soc. Lond.* B **264**, 69–75.