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The role of infectious disease, inbreeding and mating preferences in maintaining MHC genetic diversity: an experimental test

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SUMMARY

In house mice, and probably most mammals, major histocompatibility complex (MHC) gene products influence both immune recognition and individual odours in an allele-specific fashion. Although it is generally assumed that some form of pathogen-driven balancing selection is responsible for the unprecedented genetic diversity of MHC genes, the MHC-based mating preferences observed in house mice are sufficient to account for the genetic diversity of MHC genes found in this and other vertebrates. These MHC disassortative mating preferences are completely consistent with the conventional view that pathogen-driven MHC heterozygote advantage operates on MHC genes. This is because such matings preferentially produce MHC-heterozygours progeny, which could enjoy enhanced disease resistance. However, such matings could also function to avoid genome-wide inbreeding. To discriminate between these two hypotheses we measured the fitness consequences of both experimentally manipulated levels of inbreeding and MHC homozygosity and heterozygosity in semi-natural populations of wild-derived house mice. We were able to measure a fitness decline associated with inbreeding, but were unable to detect fitness declines associated with MHC homozygosity. These data suggest that inbreeding avoidance may be the most important function of MHC-based mating preferences and therefore the fundamental selective force diversifying MHC genes in species with such mating patterns. Although controversial, this conclusion is consistent with the majority of the data from the inbreeding and immunological literature.

1. INTRODUCTION

Gene products of the major histocompatibility complex (MHC) play a critical role during immune recognition by serving as antigen receptors that bind peptide fragments for cell-surface presentation to T lymphocytes (Babbitt *et al.* 1985; Bjorkman *et al.* 1987). Each MHC molecule binds a specific subset of peptides (9–20 amino acids in length) representing the intracellular degradation products of both self and non-self proteins. Each T cell expresses a single receptor that is specific for a specific type of MHC–peptide structure. The T-cell receptor repertoire is drawn from a large pool of receptors (estimated at 10^{10}) generated by gene rearrangement processes that are similar to those responsible for antibody diversity (Davis 1985). Before activation, T cells pass through the thymus, where those that recognize MHC–self peptides are terminated, leaving only T cells that recognize MHC–non-self peptides. Because T-cell recognition of an MHC–peptide structure is the triggering event of the immune response, this T-cell selection process confers a primary mode of self–non-self discrimination by the adaptive immune response. The cellular and molecular mechanisms of this MHC-dependent immune recognition process have been reviewed extensively; see Rotzschke & Falk (1991), Matsumura *et al.* (1992) and references therein.

This crucial function of MHC gene products in immune recognition has led to the widely held view that the unprecedented genetic diversity of MHC genes results from pathogen-driven selection. An MHC-like immune recognition process would be expected to lead to a coevolutionary molecular arms race favouring the genetic diversification of MHC genes. This host–parasite antagonistic coevolutionary process was, in its general form, first predicted by Haldane to account for the extraordinary diversity of vertebrate cell-surface molecules (Haldane 1949). The general hypothesis was later developed by Hamilton (Hamilton 1982; Hamilton *et al.* 1990) and others; see Michod & Levin (1988) for overview papers primarily in the context of the evolutionary forces that maintain sexual reproduction. The adaptation of this process to the diversification of MHC genes was first proposed by Bodmer (1972) and has been continued by many investigators (for example Howard 1991; Slade and McCallum 1992). Pathogens are proposed to evade MHC-dependent immune recognition by mutating

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their genes encoding MHC-targeted peptides so that they are no longer presented (bound) by MHC, or recognized (bound) by T-cell clones, or both. Such MHC-dependent pathogen evolution directed against a particular MHC phenotype will be undermined when an adapted pathogen infects a conspecific host expressing a different MHC phenotype. Such host individuals will present a different subset of peptides, thereby making previous pathogen evasion events in the context of other MHC phenotypes irrelevant. As a result, this coevolutionary process would be predicted to favour both relatively rare MHC genotypes (negative frequency-dependent selection) and MHC heterozygotes (heterozygote advantage or overdominance) (Doherty & Zinkernagel 1975; Hughes & Nei 1988; Potts and Wakeland 1990; Takahata & Nei 1990; Slade & McCallum 1992; Potts & Wakeland 1993). Both types of selection can, under appropriate conditions, explain observed levels of MHC genetic diversity (Takahata & Nei 1990). The major problem with this elegant and seductive hypothesis is a general lack of empirical confirmation. There are no convincing examples of either MHC heterozygote advantage or MHC-dependent host–parasite coevolution (frequency-dependent selection), although a sufficient amount of circumstantial evidence persists to keep this pathogen-based hypothesis viable (Potts & Wakeland 1993).

If MHC homozygosity is deleterious due to increased susceptibility to infectious disease, then the evolution of reproductive mechanisms that allowed parents to preferentially produce disease-resistant, MHC-heterozygous offspring would be predicted. Mating preferences that accomplish precisely this end have been experimentally demonstrated in *Mus* (house mice), where MHC-dissimilar mates are preferred both under laboratory conditions (Yamazaki *et al.* 1976, 1978, 1988; Egid & Brown 1989) and in semi-natural populations (Potts *et al.* 1991). These MHC-disassortative mating preferences are diversity-maintaining and are sufficient to account for the majority of the genetic diversity observed in *Mus* populations (Potts *et al.* 1991; Hedrick 1992). Furthermore, recent reports of MHC-based mating patterns in humans (Ober *et al.* 1993) suggest that this trait may have some generality in mammals and possibly other vertebrates. The evolution of MHC-based disassortative mating preferences is predicted by the pathogen-driven hypotheses described above, provided there are mechanisms whereby individuals could evaluate the MHC genotypes of prospective mates. Such a mechanism has been convincingly demonstrated in both *Mus* (house mice) (Yamaguchi *et al.* 1981) and *Rattus* (rats) (Singh *et al.* 1987), in that MHC genes influence individual odours in an allele-specific fashion. Mutations in a single MHC gene alter the odour of those individuals carrying the mutation (Yamazaki *et al.* 1983). Thus, the extreme genetic diversity of MHC antigen-binding sites results not only in extensive variation in patterns of antigen presentation, but also in an extensive array of MHC-specific odour types.

An alternative, but not mutually exclusive, function for the evolution of MHC-based disassortative mating preferences is inbreeding avoidance (Brown 1983; Partridge 1988; Uyenoyama 1988; Potts and Wakeland 1990, 1993; Alberts & Ober 1993; Brown & Eklund 1994). The extreme genetic diversity of MHC genes coupled with the olfactory ability to discriminate MHC-mediated odour types by at least some mammals (all those that have been tested, including house mice (Yamaguchi *et al.* 1981), rats (Singh *et al.* 1987) and humans (Gilbert *et al.* 1986)) makes it a potentially useful system for recognizing and avoiding mating with kin (Getz 1981; Potts & Wakeland 1993; Brown 1983; Partridge 1988; Brown & Eklund 1994; Alberts & Ober 1993). For example, by avoiding mating with prospective mates who carry one or more alleles identical to those found in one's own parents, all full- and half-sib matings and half of all cousin matings will be avoided (Potts & Wakeland 1993).

This experimental study was designed to discriminate between the inbreeding and pathogen-mediated heterozygote advantage hypotheses by measuring the fitness consequences of both inbreeding and MHC homozygosity in semi-natural populations of wild-derived *Mus*. In populations we refer to as correlated, MHC-homozygosity was correlated with moderate levels of genome-wide inbreeding, as it generally would be in nature. Animals in uncorrelated populations were systematically bred to eliminate the correlation between MHC homozygosity and inbreeding. We reasoned that if the fitness consequences of inbreeding is more important than MHC homozygosity, then inbred individuals (which are also MHC homozygotes) will have reduced fitness in the correlated populations, whereas the MHC homozygotes in uncorrelated populations will show little or no fitness reduction. Alternatively, if MHC homozygosity is more important, then MHC homozygotes will show reduced fitness in both correlated and uncorrelated populations. If both inbreeding and MHC homozygosity have a similar impact on fitness, then the fitness declines of MHC homozygotes will be greater in correlated populations where both forces are acting in concert.

Perhaps the most important component of fitness for male house mice is their ability to gain and hold territories. The conventional view of the house mouse mating system is that almost all male breeding is done by territory holders (Bronson 1979). Genetic analysis of over 1500 pups analysed from our semi-natural populations by MHC genotyping (a four-allele system) shows that the paternity of all pups was consistent with one of the territorial males, confirming that subordinate males have little or no reproductive success (Potts *et al.* 1992). Consistent with these genetic data, in 41 observed matings, none involved non-territorial males (Potts *et al.* 1991).

Both inbreeding depression and increased disease susceptibility due to MHC homozygosity are expected to negatively affect health and vigour. We predicted that the ability of males to gain and hold territories may be a sensitive measure of health and vigour differences, because males compete and fight aggressively over territories. Consequently, small differences in health and vigour that might be undetectable in

non-competitive conditions would be amplified by this head-to-head competition for territories. Thus, we used territoriality as the measure of fitness for males; for females, we used failure or success in reproduction (measured at birth). We are unable to use actual reproductive success as a fitness measure because for males paternity is confounded by high levels of extra-territorial matings by females (Potts *et al.* 1991); for females, maternity is confounded by high levels of communal nesting (Manning *et al.* 1992).

2. METHODS

(*a*) *Animals*

We had two major requirements for the experimental animals. They must exhibit normal social behaviour and have well-characterized MHC regions. In our experience inbred strains of mice do not exhibit normal social behaviour (Manning *et al.* 1992*b*), whereas crosses between inbred strains and wild-caught mice do (Potts *et al.* 1991; Manning *et al.* 1992*a*). Crossing wild animals with inbred lines allowed us to preserve well-characterized MHC regions from inbred strains while retaining social behaviour described for wild populations (Bronson 1979).

Animals used in these experiments came from generations three and six of original crosses between wild-caught animals and four inbred strains (C57BL/6, BALB/c, B10.BR and DBA/1, carrying MHC haplotypes b, d, k and q, respectively) (figure 1). In the F_2 generation only mice homozygous for one of the four inbred-derived MHC haplotypes were used to continue this outbred colony. In the resulting outbred animals, half of the genome is wild-derived and the other half is derived from one or more inbred strains. The number of inbred strains constituting the inbred-derived portion of the genome was manipulated to test the relative importance of MHC homozygosity and inbreeding (figure 2). In correlated populations, MHC homozygotes were derived from a single inbred strain, whereas MHC heterozygotes were derived from two inbred strains (see figures 1*a* and 2*a*). This established a systematic correlation between inbreeding at MHC loci and genome-wide inbreeding, a condition that would be expected in natural populations that experience some inbreeding (Weir & Cockerham 1973), as is the case for *Mus*. In our breeding design this correlation involved one quarter of the genome and only loci derived from inbred strains. In uncorrelated populations this correlation was eliminated by crosses that systematically introduced uniform contributions of all four inbred strains into each individual (figures 1*b* and 2*b*).

(*b*) *Physical facility*

Semi-natural populations of mice were housed in a mouse-proof open-air barn at the University of Florida in Gainesville. Ambient temperatures ranged from below 0°C for several days in Dec. 1988 to a high of 39°C in June 1989. The floor was concrete;

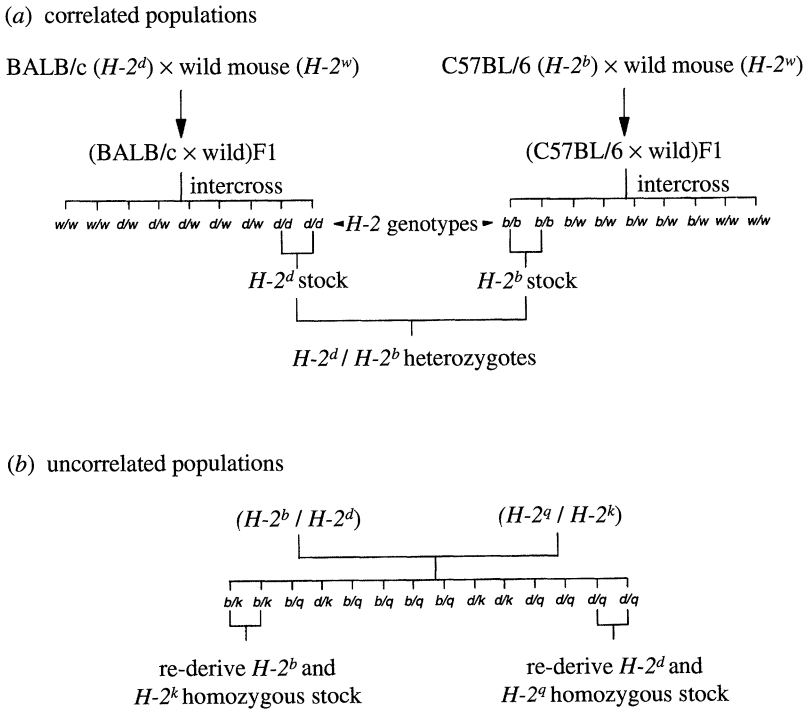

Figure 1. Breeding scheme for the production of founder mice for correlated and uncorrelated populations. (*a*) Correlated populations were produced by breeding wild trapped males with inbred females from BALB/c ($H\text{-}2^d$), C57BL/6 ($H\text{-}2^b$), DBA/1 ($H\text{-}2^q$), and B10.BR ($H\text{-}2^k$). The F1 progeny produced in this fashion were intercrossed and F2 progeny homozygous for inbred-derived *H-2* haplotypes were used as founders for *H-2* homozygous strains. *H-2* heterozygotes were produced by crossing appropriate *H-2* homozygotes. This breeding scheme is illustrated with results for BALB/c and C57BL/6. (*b*) Uncorrelated populations were produced by crossing ($H\text{-}2^b$/ $H\text{-}2^d$) heterozygotes with ($H\text{-}2^q$/$H\text{-}2^k$) heterozygotes and re-deriving *H-2* homozygous stocks.

(*a*) correlated populations

H-2	remainder of genome
homozygotes	
$H\text{-}2^{d/d}$	50% wild: 50% BALB/c
$H\text{-}2^{b/b}$	50% wild: 50% C57BL/6
$H\text{-}2^{k/k}$	50% wild: 50% B10.BR
$H\text{-}2^{q/q}$	50% wild: 50% DBA/1
heterozygotes	
$H\text{-}2^{b/q}$	50% wild: 25% C57BL/6: 25% DBA/1
$H\text{-}2^{b/d}$	50% wild: 25% C57BL/6: 25% BALB/c
$H\text{-}2^{b/k}$	50% wild: 25% B57BL/6: 25% B10.BR
$H\text{-}2^{d/q}$	50% wild: 25% BALB/c: 25% DBA/1
and so on ...	

(*b*) uncorrelated populations

all *H-2* homozygotes and heterozygotes	50% wild: 12.5% from each inbred strain

Figure 2. Origins of the genomes of founder mice for correlated and uncorrelated populations. (*a*) In correlated populations, the relative contribution of various inbred strains to the genomes of *H-2* homozygous stocks differed from that of *H-2* heterozygous stocks, resulting in an increase in genome-wide heterozygosity for *H-2* heterozygotes. (*b*) In uncorrelated populations, this bias was removed by interbreeding all the stocks and redriving *H*-2 homozygous and heterozygous founders.

sidewalls were 0.9 m high sheet metal, which prevented mice from climbing the walls. The remainder of the wall was hardware cloth with 1.25 cm grids, allowing outside air to circulate into the enclosure. The barn was 9.8 m square and was divided in two by a sheet metal barrier 0.8 m high, which allowed two independent populations to proceed simultaneously. Each population had 48 m^2 of floor space. Each side was divided by hardware cloth 0.4 m high, into eight approximately equal subsections. These barriers did not prevent passage by the mice, but did provide spatial complexity thought to be necessary for normal territorial behaviour (Mackintosh 1970). Additional complexity was provided by a 3 m spiral of hardware cloth 0.4 m high in the centre of each division. Each subsection was provisioned with Purina Rodent Chow, a poultry waterer and five widely spaced nest boxes made from clear plastic one-pint delicatessen containers with a 5 cm hole cut in the side. There was a total of 40 nest boxes and eight food and water stations per population. In addition, a hardware cloth platform 1.5 × 0.4 m was suspended from the ceiling. The platform could be reached from either end by a 0.4 m wide strip of hardware cloth that formed a ramp to the floor. The platform was provisioned with a nest but no food and water. The concrete floors were covered to a depth of 3–4 cm by wood shavings.

(*c*) *Founding stock*

All populations were started by releasing 8 males and 16 females into the enclosure. Animals were age-matched and were at least 90 days of age. None had previous sexual experience. Populations were terminated after three to four months, at which point the number of animals (approximately 125) prohibited accurate behavioural observations.

(*d*) *Behavioural observations*

During the tenure of the first correlated population (Population A) only data on male–male interactions were taken. For the next six populations (two correlated and four uncorrelated populations) an attempt was made to identify reproductive behaviour of females as well as males. Behavioural observations were made 5–7 times per week for 1–2 h at dusk, and nests were checked nearly every day. Observers worked in pairs, with one or two pairs of observers each evening. Each mouse had a unique combination of notches and holes punched in its ears. This allowed us to identify up to 100 individuals through close-focusing binoculars.

Aggressive interactions, mating behaviour, and obvious pregnancies were noted. Observations of aggressive interactions included information on where chases or fights occurred, which individuals were involved and who was the aggressor (which mouse ran and which chased). Several ten-minute focal studies of each individual were conducted where the location and behaviour of the subject was recorded at 30 s intervals. During daytime nest checks, pups were counted and nursing or attendant females or males were identified. Visibly pregnant females were noted. Records were made of females and/or males sharing sleeping nests with or without litters.

Male territoriality was determined by three criteria. Males were considered to be territorial if they regularly chased other males out of their territories, patrolled and marked boundaries, and were not seen consorting or sleeping with non-territorial subordinate males. Females were classified, in six of the seven populations, as either breeding or non-breeding. Females were considered to be non-breeding if they had not been recorded as pregnant or nursing.

(*e*) *Pathogen load*

We attempted to keep the pathogen load in our enclosure populations normal for wild, commensal, house mouse populations in Florida. We trap wild mice and bring them into our colony routinely. Because our colony is in a quarantine facility, we do not practise any special intervention to rid the colony of pathogens or parasites. In limited testing the colony has tested positive for Sendai virus, mouse hepatitis virus, extromelia, polyoma virus and *Mycoplasma pulmonis*.

(*f*) MHC *genotyping*

Animals were anaesthetized with metaphane and a 2 cm tail biopsy was taken. The tissue was frozen at −70 °C for later DNA extraction. Restriction fragment length polymorphisms from Taq1-digested genomic DNA allowed identification of all 10 MHC

genotypes. Southern blots were hybridized with a 5.8 kb EcoR1 fragment cloned from the mouse MHC class II A_β gene. Protocols for DNA extraction, Southern blotting and hybridization are detailed elsewhere (McConnell *et al.* 1988).

3. RESULTS

Three to five males in each population gained and successfully held territories. The remainder of the males became non-territorial subordinates. MHC was the genetic marker we followed for both correlated and uncorrelated populations. If disease-based selection operating through MHC heterozygote advantage was most important then MHC heterozygotes would have an increased probability of gaining territories in both correlated and uncorrelated populations. If inbreeding depression was more important, then MHC heterozygotes would have an advantage in the correlated populations, but the effect would be eliminated in the uncorrelated populations where the correlation between MHC homozygosity and genome-wide inbreeding had been eliminated. Table 1 summarizes the genome-wide inbreeding status of individuals according to MHC genotype and population. Table 2 shows the results of the experiments from three correlated and four uncorrelated populations. MHC heterozygotes enjoyed a fourfold advantage in gaining territories in the correlated population ($p = 0.02$; Fisher Exact Test) but this difference was not observed in the uncorrelated populations.

Table 3 shows that a high percentage of females gave birth in all populations and that among the females that did not give birth there was no correlation with inbreeding or MHC homozygosity.

4. DISCUSSION

In this study we measured the fitness consequences of both experimentally manipulated levels of inbreeding and MHC heterozygosity and homozygosity. We found that neither modest levels of inbreeding nor MHC homozygosity significantly influenced whether a female would give birth or not (Table 3). For males there was a detectable fitness decline associated with inbreeding (Table 2). Relative to more outbred control individuals, inbred males were less likely to gain territories. In contrast, MHC homozygosity had no detectable influence on males fitness (Table 2). These findings suggest that MHC-based disassortative mating preferences may be more important for avoiding inbreeding than for avoiding MHC homozygosity, at least in *Mus*. Because it appears that MHC genetic diversity is being maintained largely by mating preferences in *Mus* (Potts *et al.* 1991; Hedrick 1992; Potts & Wakeland 1993), these findings indicate that the functional significance of MHC genetic diversity in *Mus* may be associated more with inbreeding avoidance than with resistance to infectious disease. It is important to emphasize that the inbreeding-avoidance and disease-resistance hypotheses are not mutually exclusive. Both inbreeding and disease factors are likely to be operating; the only question is the relative contribution of these two evolutionary forces.

This striking difference in the ability of males to gain territories in the correlated populations must have been due to inbreeding depression and not selection operating directly on MHC genes. The inbreeding load that was experimentally engineered into this system is likely to be less than that found in nature because it only reflects homozygosity for alleles from inbred lines (figure 1). Many of the most deleterious alleles had been purged during the process of creating inbred strains. Consequently, all recessive lethal alleles had been eliminated as well as many of the major deleterious recessive alleles. The inbreeding load analysed in this study represents only a portion of the load in natural populations: the portion that was fixed during the creation of the respective inbred strains. The fact that it strongly affects male territoriality emphasizes the sensitivity of male fitness parameters to inbreeding and probably to any health- and vigour-influencing variable. These data are consistent with the only other studies that measured fitness consequences of inbreeding in an animal system: large inbreeding-associated fitness declines are observed in *Drosophila* (see Charlesworth & Charlesworth (1987) for review).

The inability to detect an effect of inbreeding in

Table 1. *The genome-wide inbreeding status of MHC homozygotes and heterozygotes in the correlated and uncorrelated populations*

	genome-wide inbreeding status	
populations	MHC homozygotes	MHC heterozygotes
correlated	inbred	outbred
uncorrelated	random bred	random bred

Table 2. *The influence of MHC homozygosity or heterozygosity and inbreeding on the ability of males to gain territorial dominance*

	% males gaining territories	
populations	MHC homozygotes (n)	MHC heterozygotes (n)
correlated	16 (12)	67 (12)
uncorrelated	56 (16)	44 (16)

Table 3. *The influence of MHC homozygosity or heterozygosity and inbreeding on the probability of females giving birth*

	% females that gave birth	
populations	MHC homozygotes (n)	MHC heterozygotes (n)
correlated	87 (15)	93 (15)
uncorrelated	77 (30)	78 (32)

females is not surprising for two reasons. First, high variance in male reproductive success but relatively low variance in female reproductive success is the normal condition in most animal systems. In house mice this would be expected because males compete in aggressive encounters for territories, whereas females do not compete directly for breeding rights. Second, whether females did or did not give birth is a relatively crude measure of reproductive success and could have been too insensitive to detect an actual effect. Lifetime reproductive success would be a better measure; we plan to genetically determine parentage in these populations to make this evaluation.

The experimental design of this study has two weaknesses. First, the inbreeding load was probably not normal. However, as argued above, it was probably weaker than normal; thus the main conclusion of this paper is conservative for the inbreeding effects found for males. Second, we cannot ensure that the parasites and pathogens present in our population represent normal levels found in natural populations. As described above, we attempt to keep normal pathogen loads in our enclosure populations; but because many, if not most, *Mus* pathogens have not yet been characterized this is impossible to document. Consequently, the inability to detect fitness declines due to MHC homozygosity may be due in part to an incomplete representation of *Mus* pathogens in the enclosure populations.

The data from this study suggest that, at least in *Mus*, avoiding inbreeding plays an important role in the evolution of MHC-based mating preferences, and consequently the evolution of MHC genetic diversity. Although this conclusion differs substantially from the normal immunological view, it is quite consistent with a careful review of the literature relevant to inbreeding and MHC-related resistance to infectious disease. The general conclusions in the literature are that inbreeding depression is a major fitness-reducing force that is found in essentially all species investigated, and that many species have genetic-based inbreeding-avoidance systems, often based on a single highly polymorphic locus (Charlesworth & Charlesworth 1987). A similar level of inbreeding depression in *Mus* would be predicted to drive the evolution of genetic-based disassortative mating preferences and as a consequence would favour MHC diversity, owing to MHC's central role in influencing odour types (Yamaguchi *et al.* 1981). In contrast, MHC-heterozygote advantage has never been convincingly demonstrated and MHC-related resistance to infectious disease has not been found in most cases investigated (Tiwari & Terasaki 1985; Klein 1986). The two leading exceptions to this – malaria in humans (Hill *et al.* 1991) and Marek's disease in domestic chickens (Pazderka *et al.* 1975) – are the wrong kind of disease associations because they strongly reduce fitness and they favour only one or two MHC alleles, thus resulting in reduced diversity.

In the following sections we provide brief entries to the literature on inbreeding depression, inbreeding avoidance, MHC as a kin-recognition marker, and pathogen-driven selection operating on MHC genes. Most of these areas have been recently reviewed. We take this opportunity to point out very recent findings and important ideas that are often overlooked and omitted from such discussions.

(*a*) *Inbreeding depression, inbreeding avoidance and MHC-based kin recognition*

The capacity of MHC polymorphisms to generate strong olfactory signals may have resulted in its use to avoid inbreeding through an olfaction-based kin-recognition system. The well-documented MHC-based olfactory signals of rodents provide MHC-genotype-specific information (Yamazaki *et al.* 1979). Although the molecular mechanisms responsible for the generation of these odorants are unknown, a variety of studies have directly correlated changes in odorants with specific mutations in the antigen-binding sites of MHC class I and class II molecules (Boyse *et al.* 1987). Therefore, the extensive diversity of MHC-binding sites results not only in extensive variation in the antigens presented, but also in an extensive array of odour types that are MHC-genotype-specific. Consequently, MHC-mediated odours provide genetic-based kin recognition information and disassortative MHC-based mating preferences will not only result in the preferential production of MHC-heterozygotes, but will also favour outbreeding and an increase in genome-wide heterozygosity (Uyenoyama 1988). This effect will be especially strong in small populations where prospective mating partners include many related individuals, as in *Mus* populations (Hartl & Clark 1989). Enhanced fitness due to increased genome-wide heterozygosity or inbreeding avoidance may strongly favour the evolution of MHC-based mating preferences.

In addition to this study, two other lines of evidence support the importance of inbreeding avoidance in the evolution of MHC-based mating preferences. The first is an independent demonstration that house mice, presumably through their demonstrated ability to detect MHC-genotyoe-specific odours (Yamazaki *et al.* 1979), use MHC for genetic-based kin recognition (Manning *et al.* 1992*a*). *Mus* is a communally nesting species that displays the relatively rare mammalian trait of (apparent) indiscriminate communal nursing. Such behaviour is subject to kin selection, which would promote communal nesting among kin. Restricting shared nursing to kin would decrease the probability of exploitation (Grafen 1990) and increase inclusive fitness. If MHC genes are being used as a kin recognition marker, then communal nesting partners are predicted to have high MHC similarity. When familiar sisters were available, they were strongly preferred as communal nesting parterns. However, when sisters were not available or this variable was controlled statistically, MHC-similar females were preferred as communal nesting parterns (Manning *et al.* 1992*a*). The most plausible interpretation of these data is that MHC genes are being used as kin-recognition markers to increase relatedness among communal nesting partners. These data represent the

first unambiguous demonstration of genetic-based kin recognition in any vertebrate species and consequently provide support for the inbreeding avoidance hypothesis. Such kin-recognition genes have been predicted and sought for some time (Hamilton 1964).

The second line of evidence is the observation that inbreeding depression is a strong and pervasive phenomenon of living systems (Charlesworth & Charlesworth 1987) and that many specific mechanisms have evolved throughout the plant and animal kingdoms to avoid inbreeding (Uyenoyama 1988; Blouin & Blouin 1988). Consistent with an MHC-based inbreeding avoidance system, we have recently demonstrated that the only other genetic system known to display all of the unique genetic features of MHC genes – an extreme number of alleles, ancient allelic lineages that pre-date contemporary species (trans-species evolution), extremely high sequence divergence of alleles, and high rates of non-synonymous substitutions – are plant self-incompatibility genes (Potts & Wakeland 1993). These data demonstrate empirically that all of the extreme and unusual features of MHC genetic diversity, features that are routinely cited as evidence for pathogen-drived selection, could be due to mating patterns. Disassortative mating patterns in plants have led to the only other example of such extreme genetic features and it is generally agreed that plant self-incompatibility systems evolved to reduce inbreeding.

This study and these lines of evidence suggest that, at least for *Mus*, inbreeding avoidance may be an important force leading to both the evolution of MHC-based mating preferences and the maintenance of MHC genetic diversity. MHC-based kin recognition is likely to occur in species with MHC-mediated odours, which is probably most mammals (see Brown & Eklund (1994) for recent review). MHC-mediated odour recognition in other vertebrates is perhaps less likely on grounds that the sense of smell is less acutely developed in non-mammalian vertebrates, but appropriate studies have not been done.

(*b*) *Pathogen-driven balancing selection operating on MHC genes*

We and others have reviewed the evidence for pathogen-driven balancing selection (Kiltz *et al.* 1984; Tiwari & Terasaki 1985; Klein 1986; Potts & Wakeland 1990; Takahata & Nei 1990; Slade & McCallum 1992; Hughes & Nei 1992; Potts & Wakeland 1993). The most important point to emerge is that the two types of data that could directly document pathogen-driven balancing selection – pathogen evolution in response to MHC-dependent immune recognition or MHC heterozygote advantage due to infectious disease – have not been unambiguously documented. Given this point, it is quite easy to look at the remainder of the indirect and circumstantial data and argue for or against the importance of pathogen-driven selection. For example, the most important indirect data – increased susceptibility to infectious disease based on MHC genotype – although not a prevalent feature of host–parasite interactions, has been detected (Pazderka *et al.* 1975; Lynch *et al.* 1986; Wassom *et al.* 1987; Hirayama *et al.* 1988; Nauciel *et al.* 1988; Hormaeche *et al.* 1985; Kennedy *et al.* 1986; Hamelin-Bourassa *et al.* 1989; Hill *et al.* 1991). Some view this as a meagre listing that represents a crippling blow to the pathogen-driven hypothesis; others see these studies as adequate evidence for pathogen-driven selection. We believe it is premature for any major conclusions and that new data will be required to evaluate the relative importance of pathogen-driven balancing selection operating on MHC genes. The following four observations or ideas are relatively new and are missing from most of the above reviews; they should be added to the debate.

First, there have been two recent observations that are consistent with a model of pathogen evasion of MHC-dependent immune recognition. High levels of replacement substitutions have been observed in a region of HIV (the human innunodeficiency virus) known to be used in antigen presentation by the specific MHC of the host individual from which the virus was collected (Phillips *et al.* 1991). This same region showed low substitution frequencies in virus collected from individuals with a different MHC. These data are consistent with the possibility that viral variants were favoured because they escaped MHC-based immune recognition at this epitope. Similarly, human populations in New Guinea, where there is a high incidence of human leukocyte antigen (HLA) A-11, are commonly infected with a variant of the Epstein–Barr virus which has a replacement substitution in an epitope presented by HLA A-11 (De Campos *et al.* 1993). This variant epitope is no longer presented by HLA A-11 individuals, thus suggesting that this viral variant is common because it has an advantage in HLA A-11 individuals. These two observations are intriguing and should be followed by experiments designed to test for pathogen evasion of MHC-dependent immune recognition.

Second, pathogen evasion of MHC-dependent immune recognition as the driving force behind MHC genetic diversity is rarely scrutinized. One idea that has been missing from the debate is the following possibility. Even pathogens with small genomes express many MHC-presented T-cell epitopes and each epitope has many T-cells that recognize this MHC–peptide in different ways. Consequently, it may be difficult or even impossible for any pathogen to accumulate all the necessary mutations to totally evade MHC-dependent immune recognition of all epitopes. If evasion of some but not all epitopes has little consequence for the ability of the host to respond effectively to the pathogen, then pathogen evasion will be a weak force in the evolution of MHC genetic diversity. If one were to design an immune system, a primary goal would be to make it difficult or impossible for pathogens to evade; perhaps evolution has achieved this. Again, experiments designed to test for pathogen evasion of MHC-dependent immune recognition are needed.

Third, when MHC class I-deficient mice were made, it was anticipated that they would have major deficits in their adaptive immune response and that they would be extremely susceptible to infectious disease. The big surprise is that these mice appear quite healthy in colony conditions and have shown either no or relatively minor increases in susceptibility to experimental infections (Koller and Smithies 1989; Koller *et al.* 1990; Eichelberger *et al.* 1991; Bender *et al.* 1992; Muller *et al.* 1992; Spriggs *et al.* 1992; Epstein *et al.* 1993; Grusby *et al.* 1993; Roberts *et al.* 1993). If it is difficult to document important disease susceptibilities in MHC class I-deficient mice, the task of documenting differential disease susceptibilities between MHC homozygotes and heterozygotes will be far more difficult.

Fourth, if pathogen-driven heterozygous advantage is as strong as either the inbreeding depression measured in many species (Charlesworth & Charlesworth 1987) or the MHC-based mating preferences measured in seminatural populations of house mice (Potts *et al.* 1991), then it would have been detected by now at least in humans and house mice, where there has been extensive work on MHC genes and infectious disease. This suggest that in species with MHC-based mating preferences similar to those observed in *Mus*, these mating preferences may be the primary force maintaining MHC genetic diversity and they will function primarily to avoid inbreeding.

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Discussion

A. L. HUGHES (*Department of Biology, Pennsylvania State University, U.S.A.*). Dr Potts' experiment failed to discriminate between two hypotheses: (i) mice mate disassortatively based on MHC; and (ii) mice avoid mating with their siblings, which they recognize by a variety of cues including MHC-based ones.

The second of these will not enhance polymorphism in a large natural population. To discriminate between these, one needs to conduct additional experiments. I will suggest one. A situation could be set up in which females can choose among the following: (a) a familiar full sibling, which shares neither MHC haplotype with the female: (b) an unfamiliar unrelated male which shares one or both of its MHC haplotypes with the female; and (c) an unfamiliar unrelated male which shares neither MHC haplotype with the female.

On the hypothesis of MHC-based dissassortative mating, females should prefer (a) and (c) to (b), but should not discriminate between (a) and (c). If females only avoid sib-mating, they should prefer (b) and (c) to (a) but should not discriminate between (b) and (c). If females avoid sib-mating but also mate dissociatively based on the MHC, their preference should be in the order (c) > (b) > (a).

Further experiments could address the roles of familiarity and genotype in the recognition, which Dr Potts has also failed to address.

W. K. POTTS. Dr Hughes' two hypotheses do not seem to be alternatives to each other. The first hypothesis states that mice have MHC disassortative mating preferences: the second states that mice have MHC disassortative mating preferences as well as other mechanisms to avoid sib matings. From the mating preference study suggested by Dr Hughes it seems that the question of interest is: Are familiarity or MHC-based cues more important in mating preferences? Our original study (Potts *et al.* 1991) included Dr Hughes' conditions (b) and (c); females in semi-natural populations were allowed to choose between unfamiliar unrelated males that were either MHC similar or dissimilar. They preferred MHC dissimilar males indicating that MHC-based mating preferences are used in situations not involving sibs. We avoided offering females familiar sib males due to the expectation that these males would be avoided independent of MHC cues. We agree with Dr Hughes that this is an assumption in need of testing. But we disagree with Dr Hughes when he says that MHC disassortative mating preferences that function to avoid sib matings will not enhance polymorphism in a large natural population. It has been shown that genetic-based disassortative mating preferences have similar population genetic dynamics to heterozygote advantage (Karlin, S. & Feldman, M.W. 1968 Further analysis of negative assortive mating. *Genetics* **59**, 117–136) and will be a strong selective force favouring polymorphism, independent of whether they function in conjunction with other cues to avoid sib matings. For example, plant selt-incompatibility loci which function in conjunction with other mechanisms to avoid matings with self, sibs and other close relatives display extreme genetic diversity (Potts & Wakeland 1993).