CHAPTER 17

MHC SIGNALING DURING SOCIAL COMMUNICATION

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Abstract: The major histocompatibility complex (MHC) has been known to play a critical role in immune recognition since the 1950s. It was a surprise, then, in the 1970s when the first report appeared indicating MHC might also function in social signaling. Since this seminal discovery, MHC signaling has been found throughout vertebrates and its known functions have expanded beyond mate choice to include a suite of behaviors from kin-biased cooperation, parent-progeny recognition to pregnancy block. The widespread occurrence of MHC in social signaling has revealed conserved behavioral-genetic mechanisms that span vertebrates and includes humans. The identity of the signal's chemical constituents and the receptors responsible for the perception of the signal have remained elusive, but recent advances have enabled the identification of the key components of the behavioral circuit. In this chapter we organize recent findings from the literature and discuss them in relation to four nonmutually exclusive models wherein MHC functions as a signal of (i) individuality, (ii) relatedness, (iii) genetic compatibility and (iv) quality. We also synthesize current mechanistic studies, showing how knowledge about the molecular basis of MHC signaling can lead to elegant and informative experimental manipulations. Finally, we discuss current evidence relating to the primordial functions of the MHC, including the possibility that its role in social signaling may be ancestral to its central role in adaptive immunity.

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INTRODUCTION

MHC (also known as HLA in humans and H-2 in mice) signaling mediates both immune recognition during the adaptive immune response (discussed in the previous chapter) and social signaling that enhances both the recognition of optimal mates and kin-biased behaviors.[1] Social signaling meditated by the MHC was first discovered in regards to mate preferences in laboratory mice (*Mus musculus*),[2] a full three decades after the histocompatibility functions were described by George Snell.[3] Thirty years later, social signaling via MHC has been described throughout vertebrates including mammals, birds, reptiles, amphibians and teleost fish (see Table 1). MHC social signaling has been identified in over 20 species of vertebrates and is likely the basis for a vertebrate-wide chemosensory communication system. The original observation of MHC disassortative mating preferences seems to be common, but not omnipresent in vertebrates;[4] it by no means is the only behavior facilitated by MHC, nor is it the only type of observed MHC-based mate preference. MHC signaling also facilitates cooperative behavior with kin, parent-progeny recognition and pregnancy block. In the following sections we will present the current evidence for MHC as a signal of relatedness, individuality, genetic compatibility and quality. MHC-mediated behaviors are diverse and though general patterns exist within vertebrates, the exact function of MHC-based social signaling will be species specific and highly context dependant.

SIGNALING OF MHC GENOTYPE: MOLECULAR MECHANISMS

For three decades after the discovery of MHC-mediated social singling in laboratory mice,[2] the actual mechanism of how MHC genotype was perceived in conspecifics remained a mystery. Early on it was discovered that MHC genotype could be discriminated by chemical cues detected by the olfactory system. These studies showed that mice could discriminate MHC odortypes either through training[5] or in the absence of training.[6] However, the nature of the signaling odorants remained elusive. This mystery was at least partially solved by the discovery that peptides known to bind MHC molecules also bound receptors in the vomeronasal organ (VNO).[7] It was later shown that a similar process was working in the main olfactory epithelium (MOE).[8]

The critical role of MHC-presented peptides during adaptive immune recognition is well established.[9] MHC-bound peptides are presented at the cell surface for interrogation by T cells; when the peptides are of foreign origin (e.g., from a pathogen) an immune response is initiated. The majority of MHC alleles encode unique structural aspects of the peptide binding region of the molecule and these variants provide great specificity in the peptides they present. Because there is physical correspondence between MHC allelic variants and the anchor positions of the amino acid sequence of their bound peptides, it was hypothesized that MHC peptides could serve as ligands for odorant receptors that had similar binding specificity, thus allowing information about MHC genotype to be conveyed. Physiological recordings from vomeronasal sensory neurons (VSNs) stimulated with synthetic peptides proved this to be the case.[7]

Table 1. Summary of studies investigating MHC-genotype signaling in social communication

Species	MHC-Based Mate Preference	MHC-Mediated Cooperative Behavior	Phenotype Matching System	Sources
Mammals				
House Mice (*Mus musculus*)	MHC disassortative	Female Communal Nesting	Familial imprinting	Yamazaki et al 1976,[2] 1988,[20] 2007;[21] Manning et al 1992;[22]
Bank voles (*Clethrionomys glareolus*)	MHC disassortative	Unknown	Unknown	Radwan et al 2008[23]
Malagasy giant jumping rat (*Hypogeomys antimena*)	MHC assortative	Unknown	Unknown	Sommer 2005[24]
Humans (*Homo sapiens*)	MHC disassortative	Unknown	Unknown	Wedekind et al 1995;[25] Havlicek and Roberts 2008[26]
Mandrill (*Mandrillus sphinx*)	MHC disassortative	Unknown	Unknown	Setchell et al 2009[27]
Fat-tailed dwarf lemur (*Cheirogaleus medius*)	MHC supertype-disassortative and maximal diversity	Unknown	Unknown	Schwensow et al 2008[28]
Grey mouse lemur (*Microcebus murinus*)	MHC disassortative (cryptic)	Unknown	Unknown	Schwensow et al 2008[29]
Domestic sheep (*Ovis aries*)	No MHC preference			Paterson and Pemberton 1997[30]
Birds				
Savannah sparrows (*Passerculus sandwichensis*)	MHC disassortative	Unknown	Unknown	Freeman-Galant et al 2003[31]
House Sparrow (*Passer domesticus*)	MHC assortative and optimal diversity	Unknown	Unknown	Bonneaud et al 2006[32]
Seychelles warbler (*Acrocephalus sechellensis*)	MCH maximal diversity	Unknown		Richardson et al 2005[33]
Great reed warbler *(Acrosephalus arundinaceus*)	No MHC preference			H. Westerdahl 2004[34]
Red jungle Fowl (*Gallus gallus*)	MHC disassortative (cryptic)	Unknown	Unknown	Gillingham et al 2009[35]
Peafowel (*Pavo cristatus*)	MHC maximal diversity (cryptic)	Unknown		Hale et al 2009[36]

(continued on next page)

Table 1. Continued

Species	MHC-Based Mate Preference	MHC-Mediated Cooperative Behavior	Phenotype Matching System	Sources
Reptiles				
Sand lizards (*Lacerta agilis*)	MHC disassortative	Unknown	Unknown	Olsson et al 2003[37]
Tuatara (*Sphenodon punctatus*)	MHC disassortative	Kin avoidance during territory acquisition	Unknown	Miller et al 2009[38]
Amphibians				
African clawed Frog (*Xenopus laevis*)	Unknown	Tadpole schooling	Self reference	Villinger and Waldman 2008[39]
Tiger Salamanders *(Ambystoma tigrinum*)	MHC assortative	Unknown	Unknown	Bos et al 2009[40]
Fish				
Zebrafish (*Danio rerio*)	Unknown	Unknown but kin groups grow faster than non kin groups	Familial imprinting	Gerlach et al 2007[41] and 2008[42]
Three-spined stickleback (*Gasterosteus aculeatus*)	Optimal MHC diversity	Unknown	Self reference	Aeschlimann et al 2003;[43] Reusch et al 2001;[44] Milinski 2006[45]
Atlantic Salmon (*Salmo salar*)	MHC disassortative	Schooling with kin	Self reference	Rajakaruna et al 2006;[46] Consuegra and de Leaniz 2008[47]
Chinook salmon (*Oncorhynchus tshawytscha*)	MHC disassortative	Unknown	Unknown	Neff et al 2008[48]
Arctic Char (*Salvelinus alpinus*)	Unknown	Schooling with kin	Self reference	Olsen et al 2002[49]
Brown Trout (*Salmo trutta L*)	Optimal MHC diversity	Unknown	Self reference	Forsberg et al 2007[50]
Brook Trout (*Salvelinus fontinalis*)	Unknown	Schooling with kin	Self reference	Rajakaruna et al 2006[46]
Whitefish (*Coregonus sp.*)	No MHC preference (cryptic)			Wedekind et al 2004[51]

Sources are limited to first reports and reviews. Blank boxes indicate no finding would be expected given the observed result.

Detection of Peptides in the Olfactory System

The olfactory system of mammals is anatomically divided into two regions: the main olfactory epithelia (MOE) and the vomeronasal organ (VNO). Traditionally these two organs were viewed as functioning in largely non-overlapping modalities with the VNO being specialized for detection of nonvolatile small molecules and proteins that typically signaled the sexual and social status of conspecifics (pheromones), while the MOE was thought to specialize as a general detection system for volatile substances.

The initial experiments to determine if the olfactory system was capable of detecting peptides were conducted in the VNO of mice. Leinders-Zufall and coworkers (2004) tested the hypothesis that dissociated MHC class I peptides could be detected in the VNO Two peptides known to be presented either by the H-2D^{b} haplotype (AAPDNRETF) or H-2K^{d} haplotype (SYFPEITHI) were synthesized. These peptides were applied individually to ex-vivo preparations of mouse VNO. Both peptides activated a relatively specific subset of V2R-positive neurons in the basal zone of the VNO as revealed by extracellular field potential recordings and fluorescence imaging. The VSNs responded with high sensitivity at concentrations down to 10^{-12}M.

As predicted by the hypothesis that peptides can signal MHC genotype, the peptide binding by the VSNs responded in an MHC allele-specific manner. Not only was the VSN response specific to the amino acid sequence of each peptide, but the pattern of specificity mimicked the binding properties of MHC molecules. Amino acid substitutions (underlined) at non-anchor positions (e.g., SYIPSAEKI) usually continued to stimulate the same neurons. In contrast, substitution of peptide anchor residues (underlined) with alanine (e.g., AAPDARETA or SAFPEITHA) abolished stimulation of these neurons. These VSN binding properties provide a neurophysiological basis for identifying the MHC genotype of individuals, because peptides are reverse-image "molds" of the antigen-binding site of MHC molecules. Thus, sensory receptors that detect peptides in an MHC-like fashion could in principle function as an MHC genotyping system.[10] These results point to the structural importance of peptide anchor residues in binding VSN receptors and, given the similar binding properties of MHC molecules, reveal the convergent ligand-binding properties of these unrelated molecules.

The same lab group applied the same hypotheses to the MOE sensory neurons, traditionally viewed as generalist receptors of volatile chemosignals.[8] Contrary to conventional wisdom, they discovered that nonvolatile, fluorescent tagged MHC peptides gain access to the MOE without direct nasal contact to the peptide containing fluid. Most importantly, these peptides activated neurons at subnanomolar concentrations in an allele specific fashion, similar to the patterns found in the VNO. There are, however, some important physiological differences in peptide detection between the two olfactory organs.[11] First, a different transduction mechanism is used in the MOE during recognition of peptides.[12] Second, when anchor residues are substituted with alanine (eg. AAPDARETA and SAFPEITHA), olfactory sensory neurons (OSNs) cease firing at normal stimulation concentrations, but firing resumes at higher concentrations. Third, MOE-dependent peptide recognition does not induce pregnancy block,[13] despite normal MHC odor (mating) preferences. These experiments show that discrimination of MHC genotype by the two olfactory systems is achieved with separate neurological, physiological and behavioral response pathways.

If peptides are the odorants that allow MHC genotype to be discriminated, then experimental manipulation of peptides should alter behavioral responses in a fashion

consistent with MHC-mediated behaviors. The findings that the MOE and the VNO can detect peptides in an MHC-like fashion stimulated research confirming that both mouse and stickleback fish (*Gasterosteus aculeatus*) behavior is manipulated by the addition of peptides. The experimental addition of peptides to an MHC similar odor source causes animals to respond as if it were an MHC dissimilar odor source for both mating- and odor-preferences[8,14] and pregnancy block.[7]

Signaling of MHC Genotype without Peptides

Due to the general nonvolatility of peptides,[15,16] the question has remained whether peptides can explain all of the observed MHC-mediated behavioral patterns. This question was recently addressed by experimentally removing all of the peptide components from the urine of two MHC-congenic strains of mice. Mice that had been trained to discriminate between the urine odors of these two strains could continue to discriminate using the peptide-free urine.[17] These results suggest that nonpeptide volatile odorants also provide signals conveying MHC genotype information. However, odor-training experiments can introduce confounding behavioral artifacts[18] and this result should be confirmed in a paradigm that does not use training. If these results are confirmed, making yet a third independent mechanism for identifying MHC genotype, it underscores the functional importance of this olfactory ability and the importance of the associated behavioral responses.

Though it has been shown that peptides signal MHC genotype in mammals (mice) and fish (sticklebacks), the utilization of peptides in other vertebrates is undocumented. It has been questioned weather olfaction can explain MHC-mediated behavior in birds whose olfactory prowess has long been questioned.[19] No other mechanisms have been as thoroughly tested as peptide and volatile olfaction signaling of MHC genotype and more work is needed to test whether these mechanisms drive MHC mediated behavior in other taxa.

MHC AS A SIGNAL IN INDIVIDUAL RECOGNITION

Individual recognition is an important component of social behavior. Traits that specifically signal individual identity are predicted to be genetically determined, highly variable, cheap to produce (i.e., not condition-dependent) and signal variants are expected to have equal fitness at equilibrium (reviewed in Tibbetts and Dale 2007 ref. 52). MHC is an ideal candidate gene for understanding the mechanistic bases of individual recognition because it is a genetically determined trait associated with social behavior and is extremely variable (there are 10^9 MHC phenotypes in mice[53]). MHC was hypothesized to contribute to individual recognition as early as 1975.[54] Since then, the concept of individual recognition has been invoked in many studies addressing MHC-associated cues in social signaling (e.g., ref. 17). However, many authors tacitly use different definitions of this term and do not distinguish between individual recognition in the strict sense[52] and other forms of social recognition, which can include discrimination of familiar vs unfamiliar conspecifics, kin vs nonkin, same-genotype vs different-genotype and genetically compatible vs incompatible mates. We define individual recognition as being characterized by individual specificity in three elements of social communication: signaling; signal perception and template matching by the signal receiver; and a functional response by the receiver.[55] This definition includes any case where receivers have a template of a specific individual

based on a learned signal and differs from kin-recognition where the template is based on phenotype matching (see below). Here, we review studies that have sought to characterize individual-specific MHC odortypes, which have mainly focused on MHC-correlated volatile profiles and their relation to pregnancy block and scent marking.

MHC congenic strains of mice, which share the same background genome, but have unique MHC haplotypes, are a model system with which to understand behavioral responses to individuals of same- or different-haplotype at a single locus. One extrapolation from studies demonstrating MHC haplotype-dependent behavior[17] in congenic strains is the possibility that, in outbred populations where MHC allelic polymorphism is likely to be very high, MHC phenotypes would be key mediators of individual recognition. For example, it has long been understood that MHC congenic strains have unique volatile organic compound signatures that are used in chemical communication.[15] More recently, several groups have identified suites of volatile organic compounds that are regulated by MHC odortypes.[15,16,56] As predicted by a model of individual recognition, some of these suites are unaffected by environmental variation;[57] furthermore, volatile profiles from MHC congenic mice activate overlapping but distinct subsets of neurons in the mouse main olfactory bulb.[58] The authors of such studies in congenic strains often conclude that the physiological machinery is in place for volatile profiles to mediate individual-specific behaviors (e.g., ref. 57). However, counter-part experiments using outbred wild mice in a more ecologically realistic setting are lacking. Given that some genotypes will inevitably be shared between individuals, more naturalistic work is needed to understand how these volatile signatures function as signals of individuality (as defined above) or as signals of relatedness or genotype.

Pregnancy Block

Pregnancy block, also known as the Bruce effect, occurs when recently mated female laboratory mice are exposed to the odors of an unfamiliar male.[59] Upon exposure to an unfamiliar male odor, prolactin release from the anterior pituitary in the mated female is suppressed, resulting in pregnancy failure, reabsorption of the fetus and the onset of estrus.[60] The signal responsible for pregnancy block is considered to be individual specific because the unfamiliar male and the mate both express odors capable of inducing pregnancy block. Thus, females have to learn the identity of their mate (i.e., form a memory) in order to suppress pregnancy block upon perception of the mate's odors.

Pregnancy block can be induced by the presence of an unfamiliar male or simply his soiled bedding or urine and direct physical contact with the odorant seems necessary.[60] However, in at least one case volatiles alone (i.e., no direct contact) can induce pregnancy block.[61] The memory developed during pregnancy block is dependent on activation of sensory neurons in the VNO; however, the specific chemical constituents that bind receptors in these neurons have proven difficult to find. Three different classes of molecules associated with individual odors have recently been investigated: MHC and MHC peptides, major urinary proteins (MUPs) and volatiles. Peele and colleagues recently investigated the relative roles of MUPs and volatiles.[62] They found that low molecular weight fractionations (which excludes MUPs) from urine were more effective in blocking pregnancy than those of high molecular weight, suggesting a role of volatile compounds in the odor. However, the low molecular weight fraction from the unfamiliar male resulted in only 50% pregnancy block, as opposed to 90% pregnancy block via unfamiliar male whole urine. Moreover, a recent study called these findings into doubt by showing that,

contrary to Hilda Bruce's original finding, urine from castrated or juvenile males was sufficient to induce pregnancy block. These results suggest that, although volatiles can contribute to the occurrence of pregnancy block, they are not necessary to induce it.[63]

MHC-associated odors have also been shown to be sufficient to induce pregnancy block in several studies, implicating it's involvement during individual recognition. These odors were originally observed to block pregnancy when unfamiliar males differing only at the MHC could induce pregnancy block.[61] Since then, searches for an MHC-odortype mechanism have targeted MHC molecules themselves, MHC peptides and possible associated volatiles. MHC peptides were the first specific odorant found to induce pregnancy block[7] (see above).

The finding that sensory neurons in the VNO respond selectively to MHC peptides was biologically validated by demonstrating the role of peptides in producing pregnancy block.[7] As predicted, it was found that pregnancy block upon exposure to MHC peptides from an unfamiliar, MHC-dissimilar male was equally effective as exposure to whole urine from an unfamiliar, MHC-dissimilar male. In this case, the peptides had to be delivered on a urinary background (regardless of whether the urine was from a familiar or unfamiliar male). A more recent study, however, found that peptides alone (administered more frequently than in ref. 7) were sufficient to induce pregnancy block.[63] These studies show that the suite of peptides presented by an individual's MHC molecules can, when excreted in urine, be used as odorants in chemical signaling. Because of the large diversity of MHC haplotypes in a population, there is potential for individual specific odortypes simply in excreted MHC peptides. Such odortypes are detectable by VSNs that have binding specificity for these peptides similar to that of MHC molecules.[7] Where these peptide signals originate, however, remains to be found. Surprisingly, there is disagreement about whether peptides can be found in mouse urine.[7,60,64] Peptides have not been reported in other mediums of chemical communication such as saliva, tears, or skin excretions, but we are not aware of any directed searches for peptides in these secretions.

Although MHC peptides are clearly sufficient to induce pregnancy block in inbred mice, it should be noted that the experiments described above do not demonstrate individual recognition in a strict sense. Because peptides from an unfamiliar male with the same MHC genotype as the female's mate would not be expected to induce pregnancy block, MHC peptides in the context of pregnancy block might be more likely to signal the presence of an unfamiliar male. If individuality is perceived during pregnancy block, it would likely be conveyed via coupling with sensory neurons activated by the urinary background and neurons in the VNO have been found to be capable of discriminating individual mice of the same laboratory strain.[64] Finally, while pregnancy block provides an attractive system in which to test hypotheses concerning social signaling and behavior, the system is ultimately hindered by the fact that the adaptive significance of pregnancy block, which is only observed in certain laboratory strains of mice, has not been determined for natural populations. It has been suggested that the Bruce effect functions to prevent infanticide from males who have recently displaced the dominant, territorial male.[4,65]

Scent-Marking

In addition to the MHC, growing evidence indicates that major urinary proteins (MUPs) are another chemical signal critical to social communication and individual recognition in mice. MUPs are protein pheromones encoded by a polymorphic, multi-gene family. In a series of experiments, the laboratory of Jane Hurst has tested the relative

roles of MUPs and MHC in individual recognition in mice using a scent-marking behavioral paradigm. First, it was shown that wild-derived males presented with a scent mark from another male expressing a different MUP-type will investigate and counter-mark the marks significantly more than the control.[66] Second, it was shown that scent-marks associated with MHC haplotype (in MHC-congenic strains) were not necessary or sufficient to influence investigation time of male mice of congenic MHC strains. Rather, investigation time was increased only when the stimulus odor differed from the genomic background of the test animal.[67] A third experiment tested whether wild female mice could discriminate between scent marks from congenic males whose MHC and MUP genotype were controlled. Results showed that females could discriminate between individual males only when the males differed with respect to MUP haplotype; females could not discriminate between individual males that had the same MUP haplotype and could not discriminate between males that had different MHC haplotypes.[68] These three experiments indicate that, in the context of scent-marking and countermarking, MUP genotype and not MHC genotype, is the greatest determinant of individuality in urinary odors. However, it should be noted that in light of previous research, it is anomalous that the mice in these experiments did not discriminate between urinary odors that differed with respect to MHC genotype.[67,68] Previous studies have documented the ability of either MHC-congenics (e.g., ref. 5) or wild-derived mice[69] to distinguish urinary odors that only differed genetically at the MHC.

Because MUPs are likely to be polygenic, polymorphic signals only in a few rodent species it is unlikely that the functions discovered in *Mus* will have generality across vertebrates. The results from the Hurst group studies suggest that there are key differences in signals that are conserved across taxa (e.g., MHC) and signals that are species-specific (e.g., MUPs) for the identification of individual conspecifics.[68] They also reveal the curious finding that signals of individuality are limited to specific behavioral interactions.

Taken together, the individual recognition studies reviewed above show that MHC may play an important role in individual recognition in certain instances (for example in pregnancy block), but also indicate that they may not be used for individual recognition in the strict sense. Many of the studies focusing on individual recognition and the MHC have utilized congenic strains of mice, which provide a unique opportunity to study the role of a single locus or haplotype in chemical communication. However, the use of inbred stains of animals may limit our broader understanding of behavior and ecology, as 60 years of domestication has modified their behavior.[70] So, more studies will be needed to determine the role of MHC in individual recognition in outbred populations; we know of no such examples except for the aforementioned examples from the Hurst lab.

MHC AS A SIGNAL IN KIN RECOGNITION

Kin recognition using polymorphic genetic systems allows individuals to engage in behaviors specific to kin or nonkin. An individual's fitness is a product of both its own reproductive success (i.e., direct fitness) and the reproduction of close relatives (i.e., indirect fitness); thus, proper identification of kin facilitates cooperation (or at least decreased antagonism) with relatives and promotes behaviors that increase fitness.[71] Additionally, recognition of kin allows for the prevention of inbreeding, and therefore reduces the homozygous expression of deleterious recessive alleles. In order for a genetic system to be used accurately to recognize kin, it must contain enough allelic polymorphism to allow

discrimination between related and unrelated individuals. Kin recognition systems that can discriminate among a range of different-degree relatives have been reported.[72] MHC is the most polymorphic genetic system in vertebrates[4] and has long been considered to play a role in kin recognition by mediating cooperation,[1] parent-offspring identification[73] and mating preferences that prevent inbreeding.[74]

Two major phenotype matching mechanisms exist for MHC-based kin recognition within vertebrates (Fig. 1). The first is a self reference system in which individuals use their own MHC odortype as a template to recognize other individuals as kin.[39,43,46,49,50] The second is familial imprinting where individuals imprint upon the MHC odortypes of kin early in development and afterwards apply the learned MHC signals to unfamiliar individuals.[20,42,75] The degree to which familial imprinting and self reference systems identify kin differ remarkably (Fig. 1). Only familial imprinting systems can identify kin that do not share odortypes with a focal individual. However, the ability to recognize kin that do not share odortypes also allows for the false recognition of unrelated individuals as relatives; this could occur in mixed litters where odortypes produced by half siblings are based on haplotypes from an unrelated individual. Both phenotype matching systems can be used to identify kin through odortypes based on either specific MHC haplotypes (both haplotypes providing a specific odor) or by odortypes based on a blended odor of both haplotypes. For example self reference systems recognize either 25 or 75% of full siblings depending on whether specific haplotypes are recognized or only the genotypic odor of the blended haplotypes[76] (Fig. 1). Currently few studies have been conducted to determine the specifics of phenotype matching systems used in nature and more research is needed to determine the relative prevalence of familial imprinting vs self reference systems and the nature of the odortypes (specific haplotypes or blended genotypes) used. Interestingly, the two systems most described in nature are familial imprinting on haplotypes and self reference based on blended genotype odors which are the best and worst of the theorized kin recognition systems respectively (Fig. 1). Regardless of the phenotype matching system used, kin recognition is likely one of the major functions of MHC-mediated signaling and the very existence of familial imprinting is evidence supporting this hypothesis because kin recognition is the only function that is enhanced by familial imprinting; self reference will be superior for functions involving genetic compatibility, individuality, or quality.[65]

Phenotype matching systems can identify more kin if multiple polymorphic unlinked loci are used, presuming a match at any locus is a signal of relatedness.[77] Though the impact of multiple unlinked loci has minimal impact on familial imprinting systems it has profound consequences on self reference systems, where multiple loci dramatically improve kin recognition (Fig. 1). Within both teleost fishes and amphibians, taxa where self reference systems are common, the MHC is not inherited as a single unit but rather as two or four separate unlinked loci.[78,79] Whether this is coincidence or represents evidence that the inefficiency of self reference systems favors translocations that breakup the MHC linkage group will await more phylogenetic data. Within both teleost fishes and amphibians it has been shown that MHC Class II genes are sufficient, but not necessary, for kin recognition. It has been proposed that other unlinked MHC genes provide additional information used in kin recognition.[46,49] Likewise, in house mice it has been observed that when MHC signals of relatedness are controlled for, signals from a different polymorphic locus (MUPs, see below) can also be used as signals of relatedness. In nature, it is highly likely that both MUPs and MHC are utilized for kin recognition in tandem.[80]

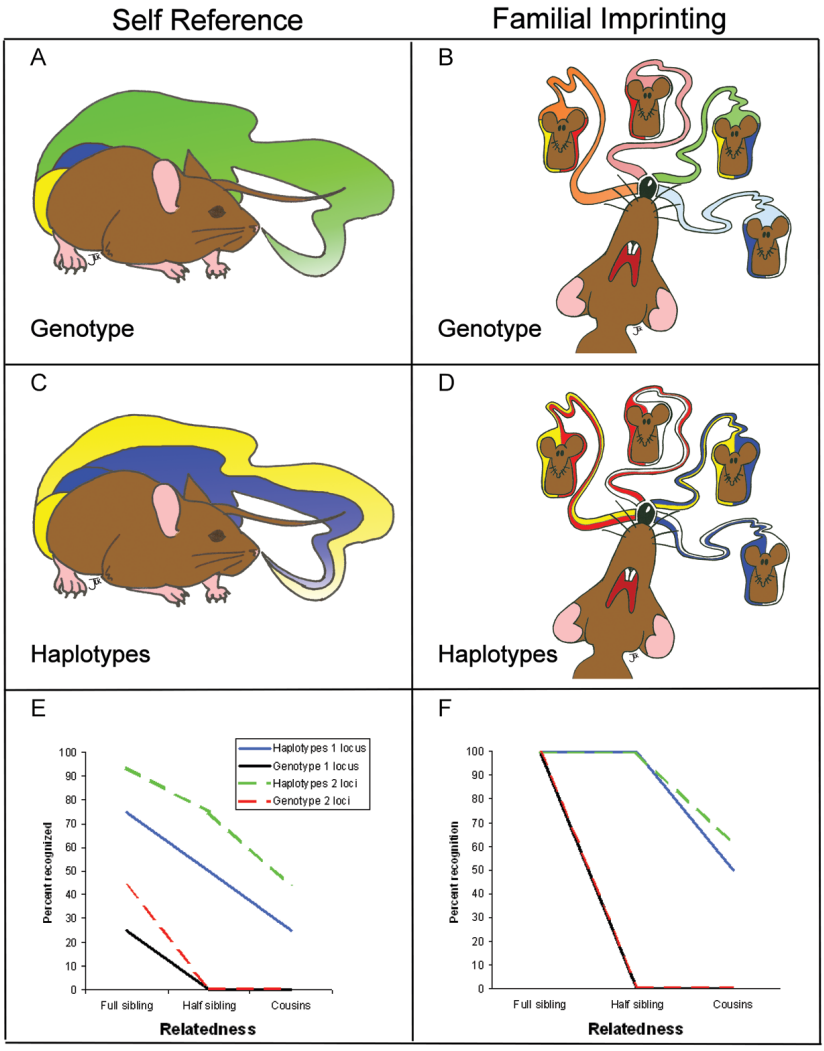

Figure 1. Possible phenotype matching systems using MHC-based odors and their effectiveness for the recognition of kin. Two kin recognition mechanisms that exist in nature are self reference (A, C, E) and familial imprinting (B,D,F). Phenotype matching can be based on genotypes (i.e. blended haplotype odor), or on haplotypes (i.e. allele-specific odors). Self reference is based on odors associated with an individual's own genotype (A) or both haplotypes (C). Familial imprinting is based on odors associated with the genotypes (B) or haplotypes (D) present in the natal nest (e.g. parents or siblings). The prevalence of these systems in nature is largely untested; current evidence suggests that the primary phenotype matching system in mice is haplotype-based familial imprinting (D). The effectiveness of each phenotype matching system for recognizing three classes of kin are plotted for one or two unlinked polymorphic loci (E & F). MHC haplotypes are inherited as a linked locus or as multiple unlinked loci depending on taxa. Each point represents the percentage of full siblings, half siblings, and cousins an individual would be able to recognize (points are connected by lines to help visualize patterns). Haplotype-based mechanisms are almost always superior to genotype mechanisms for kin recognition. Adding loci to self reference systems improves kin recognition more than in familial imprinting systems. Familial imprinting (F) generally allows an individual to recognize more kin than self reference (E). Models assume that all individuals are heterozygous, that no alleles are shared between unrelated individuals and that all combinations of parental genotypes are found within litters. (Illustrations by J.L.K; graphic design by Linda Morrison).

Cooperative Behavior

Proper identification of kin can result in cooperative behaviors between relatives; MHC mediated signaling has been shown to both promote cooperation and deter antagonism between individuals (Table 1). Schooling is an important cooperative behavior in fish and tadpoles that results in enhanced foraging and predator avoidance. Several salmonid species along with the African clawed frog (*Xenopus laevis*) have been shown to preferentially form schools with relatives that share MHC haplotypes[39,46,49] and it has been previously shown that kin-based schools have higher survival rates and larger territories.[81] A second MHC-mediated cooperative behavior has been documented in house mice; female mice communally nest and nurse offspring and it has been demonstrated that females preferentially nest with familiar sisters. When no familiar sisters are available, they preferentially nest with MHC-similar females.[1] Finally, competition over territories is fierce in many species of vertebrates and can result in serious injury; evidence suggesting that MHC signaling prevents territorial competition between kin has recently been demonstrated in tuataras (*Sphenodon punctatus*).[38] Scores of other kin-based cooperative behaviors have been documented within vertebrates and it is quite probable that we have only just begun to document those that are mediated by MHC signaling; however, it is not our intent to imply that all cooperative behaviors will be MHC-mediated. In fact, the precision of kin recognition systems will be enhanced as more polymorphic systems are used in signaling.

Parent-Progeny Recognition

Parent-progeny recognition prevents the expense associated with parental investment into unrelated individuals. This is especially true under conditions of communal living or in systems that involve extra-pair matings. Under these circumstances an identification system that could ensure parental care was only provided to genetic offspring would be highly adaptive and many such systems have been documented.[82] Female house mice nest communally and are therefore at risk of providing parental care to unrelated pups. Yamazaki and others[73] showed that female house mice can identify pups with which they share an MHC haplotype from congenic pups (genetically identical individuals with the exception of MHC type). Pups at the age of 15-21 days were also capable of recognizing and preferring their parents bedding to that of a MHC dissimilar congenic individual. This preference was reversible by cross-fostering, again showing the role of familial imprinting within MHC signaling in house mice. Currently this study offers the only evidence that MHC-mediated signaling is involved in parent-progeny recognition and though it was conducted with inbred strains of mouse, it reveals the potential of MHC signaling in nature.

Inbreeding Avoidance

Degradation of fitness due to inbreeding is a result of increased homozygosity of deleterious recessive alleles that are identical by descent. These alleles combine more frequently when related individuals reproduce compared to outbred matings. Early assessment of the fitness costs of full-sibling level inbreeding within vertebrates (mice) have been conducted and early studies showed a 10% decline in litter size.[83,84] However, these experiments only measured litter size reductions and they failed to assess the fitness

consequences of the inbred progeny in their natural context. In an experiment where the fitness impacts of a single generation of full-sibling inbreeding were assessed under seminatural conditions, it was found that outbred male mice had five-fold higher fitness than inbred males, with the consequences effectively approaching lethality for inbred sons. Daughters suffered an additional 20% reduction in fitness compared to previous assessments.[85] Likewise cousin-level inbreeding was shown to reduce male fitness by 34% and when the infectious agent *Salmonella* was present in the populations, the fitness decline in males was 57%.[86] Since the true negative consequences of inbreeding were only revealed under direct competition within a seminatural environment, we now refer to this experimental system as a phenotron because it allows the observers "to see" the true fitness consequences (phenotype) of a treatment. Disassortative MHC-based mating preferences function as a mechanism of inbreeding avoidance due to their highly polymorphic nature. Only closely related individuals are likely to share MHC haplotypes; thus a mating preference for MHC-dissimilar individuals will decrease the likelihood of inbreeding. The extent to which inbreeding can be avoided is dictated by whether a self reference or familial imprinting mechanism is utilized by a particular species.[76]

An indirect piece of evidence supporting MHC haplotype based familial imprinting and inbreeding avoidance within house mice has come from a study by Sherborne and colleagues.[80] This experiment investigated the relative importance of MHC and MUPs in mediating inbreeding avoidance behavior and its conclusion was that MHC is not involved in inbreeding avoidance behavior. House mice were released into seminatural enclosures with only full-sibling and half-sibling counterparts; inbreeding avoidance was assessed by the proportion of full-sibling vs half-sibling matings and genetic analysis was used to determine if there was either an MHC or MUP-based signal mediating inbreeding avoidance. The data showed that although no full-sibling inbreeding avoidance occurred, mice sharing exact MUP genotypes avoided mating with each other. This led the authors to conclude that MUPs are exclusively responsible for inbreeding avoidance in house mice and that MHC plays no role. However, this conclusion is unwarranted due to a flaw in the experimental design. Specifically, prior to testing in seminatural enclosures, test animals had been caged (since birth) with other individuals that possessed MHC haplotypes that were present in the enclosures. This design unintentionally allowed MHC familial imprinting to occur on all of the tested haplotypes; thus, animals upon entering the enclosures found themselves surrounded by individuals that would all be recognized as relatives by MHC-based systems. This situation forced the mice to make mate choices based on other non MHC cues and they utilized MUPs, preferring to mate with individuals that did not share exact genotypes. These results suggest MUPs are utilized in mate choice, but contrary to the conclusions of the paper, the design does not allow for the exclusion of a role for MHC. Furthermore, MUP-based mating preferences are based on self reference and not familial imprinting,[80] thus they do not offer the same protection against inbreeding that familial imprinted MHC preferences do.

MHC AS A SIGNAL OF GENETIC COMPATIBILITY IN MATE CHOICE

Genetic compatibility, broadly defined, refers to the degree to which an organism's genes, (both within and between haploid genomes), interact to increase or decrease fitness. Consequences of genetic incompatibility include inviable offspring (e.g. between species mating), severely reduced fitness (e.g. inbreeding), and incremental degradation of fitness

associated with the combination of incompatible alleles (e.g. MHC homozygosity). The fitness consequences of genetic compatibility might be so severe that finding a mate with the "right genes" to compliments one's own genome provides more indirect benefits than finding the "best genes" within high quality individuals.[87] In order to make MHC-based mate choice (or gamete fusion[88,89]) decisions in regards to genetic compatibility, individuals must possess the means to assess their own MHC types (see section on phenotype matching systems above). MHC-mediated odors readily signal information about the genetic compatibility between mates, and MHC-disassortative mating preferences (Table 1) lead to the production of offspring with compatible genotypes both at the MHC and throughout the genome.[76] The mechanisms of MHC-mediated genetic compatibility described below are MHC heterozygote advantage, offspring harboring different MHC genotypes than their parents (moving target) and the avoidance of inbreeding.

Heterozygote Advantage/Superiority

MHC-disassortative mate preferences by their very nature produce MHC heterozygous offspring, which are hypothesized to have superior immunocompetence.[90,91] Multiple lines of evidence now support the fitness-enhancing role of MHC-heterozygosity.[92-98] It was initially argued that MHC heterozygotes would have an advantage (overdominance) because they could present a wider variety of peptide antigens to the immune system making them more likely than MHC-homozygotes to recognize and mount an immune response against disease-causing agents. However, this mechanistic hypothesis has largely been rejected since experimental infections with single pathogens reveal that heterozygotes do not generally have an advantage over both homozygotes.[99] An alternative mechanism postulated that heterozygote advantage emerges over multiple infections because resistance is generally dominant and heterozygotes will benefit from the resistance profile of each allele, which masks some of the susceptibilities of each allele. This hypothesis was experimentally confirmed by laboratory-based experiments using coinfections with parasites having opposite MHC resistance/susceptibility profiles, which demonstrated that heterozygotes are more fit than either homozygote.[99] Recent studies on wild salmon[100] and vole[101] populations demonstrate that MHC heterozygotes have increased fitness under natural conditions of multi-parasite infection as well. The fitness enhancing nature of MHC heterozygote advantage in laboratory and natural settings is an example of the adaptive significance of MHC mediated signaling.

Moving Target

In addition to heterozygote advantage, selection could also favor MHC-disassortative mate preferences if the offspring genotype provided a moving target against pathogen adaptation, causing pathogens adapted to either parent to be at a disadvantage in progeny that are MHC-dissimilar to both parents.[102] This hypothesis predicts that pathogens evolve to partially escape MHC-mediated immune recognition and that MHC-dissimilar offspring are more fit than their parents when challenged with parent-adapted pathogens. Like heterozygote advantage, mate choice decisions driven by moving target processes function to maximize genetic compatibility and are thus most effectively achieved using an MHC, self reference phenotype matching system.

Numerous examples highlight the capacity of pathogens to rapidly adapt to escape MHC-mediated immune recognition.[103-110] There has been one experimental study designed

to test the other prediction of the moving target hypothesis—that MHC-dissimilar offspring will be more fit than their parents when challenged with parent-adapted pathogens. MHC did influence the trajectory of adaptation by a fungal pathogen (*Cryptococcus neoformans*), but the large virulence increase in postpassage pathogen lines showed no specificity for the host MHC genotype of passage.[111] The most likely explanation is that this pathogen is a generalist that infects most birds and mammals. The passages in mice therefore selected for adaptations to "mouseness", which likely swamped any adaptations to MHC. Future passage studies should use pathogens specialized on the host of passage.

There is anecdotal evidence from human studies demonstrating the importance of offspring genetic diversity in reducing the probability of mother-to-child-transmission of chronic infectious disease agents (e.g., HIV-1[112,113]), and suggest that there would be a significant selective advantage to mate choices that promoted genetic diversity in offspring. There is also evidence linking increased HLA dissimilarity between mother and offspring with significantly reduced chances of vertical transmission of HIV-1[114,115]). The extent of pathogen adaptation during chronic infection of the parent and its impact on mother-to-child transmission dynamics was not addressed in the above studies. Despite this, they do support the possible role of MHC- disassortative mate preferences in producing offspring of higher quality that are more resistant to infection by chronic parasites of their parents.

Optimal MHC Heterozygosity

MHC-disassortative mate choice may carry a cost if maximal MHC diversity in offspring is not optimal. For instance, during the process of negative selection in the thymus, T cells with high affinity for MHC-peptide complexes are instructed to terminate themselves via apoptosis.[9] It follows then that MHC diversity may have an upper limit beyond which the fitness benefit of having multiple ways to present peptides from foreign invaders is offset by the cost of an increasingly limited T-cell repertoire.[116] If such a fitness cost exists, then it will have important implications on the evolution of MHC disassortative mating preferences. Indeed, it has been observed that individuals with intermediate versus maximal MHC diversity harbor lower parasite burdens in experimental infections.[117] Additionally, it was recently shown that intermediate and not maximal levels of MHC diversity lead to significantly higher lifetime reproductive success in stickleback offspring.[118] Thus, it seems that maximum MHC diversity can be a costly trait.

If intermediate rather than maximal MHC diversity is optimal then an MHC-typing system could allow individuals to "optimize" the MHC diversity within their offspring. Studies with sticklebacks have shown that females are in fact capable of such quantitative estimates of MHC diversity (also known as allele counting).[119] Additionally, by estimating the extent of intra-individual MHC class IIB allele diversity within a population, it was also demonstrated that individuals with intermediate rather than maximal MHC diversity were most frequent, indicating selection for intermediate levels of MHC diversity. Subsequent experimental findings in sticklebacks[43] and brown trout[50] suggest that much of the selection for individuals with intermediate MHC diversity derives from female preference for MHC-dissimilar mates. Together, these studies indicate that maximal MHC diversity is not always optimal and that female preference for MHC-dissimilar mates is a primary driving force behind selection for the production of individuals with intermediate rather than maximal MHC diversity.

Inbreeding Avoidance

Though inbreeding avoidance has already been covered within the kin recognition section it is important to stress that it also falls under the umbrella of MHC as a signal of genetic compatibility. In fact, inbreeding avoidance may be the single most adaptive result of MHC-disassortative mating preferences in many species of vertebrates, as both sibling and cousin level inbreeding have been found to have devastating effects on the fitness of offspring.[85,86] In addition, as covered in the evolution of MHC section below, growing evidence suggests that MHC mediated kin recognition to avoid inbreeding may have been the ancestral function of MHC molecules, which were later co-opted for use in the adaptive immune system.[120]

MHC AND SIGNALS OF QUALITY IN MATE CHOICE

In contrast to MHC-mediated signals that directly convey MHC genotype information (relatedness, compatibility or individuality), the disease resistance functions of MHC can also influence social signalling by modulating the expression of secondary sexual characters. Only high-quality, disease-resistant individuals should be able to invest in costly, sexually selected advertisements,[121] thus creating a correlation between MHC genotype and these condition-dependent traits (Table 2). By endowing an individual with genetic resistance to parasites, MHC genotype can indirectly influence signals of quality by allowing more physiological resources to be devoted to signaling rather than to the immune response.[122] von Schantz and colleagues[123] were the first to report an association between MHC and a sexually selected trait; they found that spur length in male pheasants (*Phasianus colchicus*) was correlated with fitness and dependent on MHC genotype. In a study on great snipes (*Gallinago media*), females preferred males carrying specific MHC allelic lineages. Males with these genotypes were also larger and females of this species are generally known to favor larger males.[124] A study in white-tailed deer (*Odocoileus virginianus*) found that MHC divergent heterozygous males had larger antlers and body size, which was correlated with lower abundance of abomasal nematodes.[122] Finally, a study

Table 2. MHC correlations with secondary sexual traits and mating preferences

Species	MHC Correlation with Mate Preference	MHC Correlation with Traits of Quality	Sources
Great snipe (*Gallinago media*)	MHC allele-specific preference	Body size	Ekblom et al 2004[124]
Peafowl (*Pavo cristatus*)	MHC heterozygosity	Train length	Hale et al 2009[36]
Pheasants (*Phasianus colchicus*)	MHC genotype	Spur length	von Schantz et al 1996[123]
White-tailed deer (*Odocoileus virginianus*)	MHC divergent heterozygotes	Antler and body size; reduced parasitism	Ditchkoff et al 2001[122]

on a canonical sexually selected trait, trains in male peacocks (*Pavo cristatus*), showed that the train length reflects genetic diversity at the MHC.[36] The above examples show that MHC-genotype can influence the expression of secondary sexual traits that are used as signals of quality. However, MHC-genotype itself is not necessarily used in the signal.

An alternative way that MHC-genotype can indirectly influence the expression of secondary sexual characteristics is if MHC social signals are themselves costly to produce. This hypothesis has recently been tested by the laboratory of Manfred Milinski, which identified the first example of condition-dependent MHC signaling.[125] They had previously shown that female three-spined sticklebacks prefer males with optimal, rather than maximal, MHC allelic differences (relative to her own genotype) and that this mate choice is mediated by excreted MHC peptides (discussed above).[14,44] Now, they show that females do not send this signal at all and that, remarkably, males only send this signal when they are in the reproductive state. These data suggest that MHC signaling is not simply a byproduct of MHC-peptide presentation, but that it is actively regulated in a fashion consistent with it being a costly signal. The authors suggest that shedding MHC-peptide complexes will create localized deficiencies of this critical immunological component and therefore represents a trade off between immune defense and social signaling.[125]

MHC-mediated signals of quality may allow an individual to gain either direct benefits for themselves or indirect genetic benefits for their offspring. Avoidance of parasitism is perhaps the most likely direct benefit of MHC-mediated mate choice. Social behaviors are an opportunity for parasites to transmit to new hosts; in turn, hosts will gradually develop behavioral mechanisms to avoid parasites.[126] Individuals of a particular MHC-genotype may be resistant to local parasites at any given time and choosing such an individual as a mate would provide a direct benefit of reduced risk of parasitism. Although there are several examples of mate choice for parasite-free individuals,[127-129] there are surprisingly few examples of studies that link MHC-dependent resistance to pathogens and subsequent mate choice.[117]

MHC EVOLUTION: WHAT ARE THE PRIMORDIAL FUNCTIONS?

Since the immune recognition function of MHC genes in adaptive immunity was discovered far earlier than MHC-mediated behaviors, and since it was so central to the complex system of vertebrate adaptive immunity, it was initially assumed that MHC-mediated behaviors were a derived function. However, Brown argued that since kin-selected behaviors (inbreeding avoidance and kin-biased cooperation) are present in the ancestral lineages leading to vertebrates and that adaptive immunity is a derived character in vertebrates, it is most parsimonious to hypothesize that MHC-mediated kin recognition functions were primordial.[74] This controversy continues to this day.

Boehm has recently written a tour-de-force, synthetic review that evaluates self and nonself recognition systems that exist across plants, fungi and animals, with a special emphasis on how quality recognition is maintained in the face of the rapid diversification of these highly polymorphic systems.[120] Quality control (the ability to accurately discriminate between self and nonself) is of particular importance in immune recognition systems that must achieve self tolerance to protect against auto-immune disease.[120,130]

Jawless fish are the one lineage of vertebrates that appear to have a non MHC based adaptive immune recognition system.[131,132] A high diversity of lymphocyte receptors in this group is created by combinatorial assembly of receptor modules, but the critical difference

from other vertebrates is that there is no junctional diversity created by mutagenic joining mechanisms.[133] Thus, the lymphocyte receptor repertoire for jawless fish is predictable and self tolerance could be achieved by Darwinian selection for self-compatible receptor modules.[120] In contrast, jawed vertebrates achieve higher lymphocyte receptor diversity by the mutagenic VDJ combinatorial joining process, which creates the problem of unpredictable receptor specificities that can lead to auto-immunity. These potentially harmful receptors are eliminated during the evaluation of lymphocytes receptors in the thymus of jawed vertebrates. Boehm argues that it seems unlikely that an MHC-peptide presentation system could emerge de-novo to create the modern jawed vertebrate immune recognition system, which allows self-tolerance in the face of somatic generation of unpredictable lymphocyte receptors. It would be far more likely that a pre-existing MHC-peptide kin recognition system could be co-opted for immune recognition.[120] Discovery of the MHC homologues and their function in jawless fish offers one of the most promising approaches for discriminating between these two hypotheses and identifying the primordial function of MHC genes. Tunicates (a close relative of vertebrates) have a highly polymorphic histocompatibility-type (fusion) locus that functions both in allo-recognition to control colony fusion and gamete fusion,[134] at least in some species.[135] It was thought that identifying the nature of this locus might clarify the early history of MHC genes. After a two-decade search the locus was identified to be a member of the immunoglobulin super family, but it appears to not have homology to MHC genes.[136-138] These findings further focus the search for primordial MHC functions towards jawless fish.

The facts that within vertebrates there are completely different mechanisms controlling adaptive immune recognition and that in tunicates histocompatibility functions are controlled by genes unrelated to vertebrate histocompatibility genes, highlight the evolutionary flexibility of how similar functions can be achieved through different genetic systems. It is currently difficult to discriminate between the different proposed primordial function of MHC genes. However, the initial assumption that immune recognition must be the primordial function of MHC genes, should no longer be the default assumption.

CONCLUSION

In this chapter we have demonstrated the significance of MHC signaling in regards to four aspects of social communication. First, studies in mice show that MHC peptides and to a lesser extent MHC-associated urinary odors, signal individuality in the context of pregnancy block. MHC does not signal individuality during mouse scent-marking, rather, a species specific signal (MUP) is used. Second, MHC as a signal of relatedness is found across vertebrates (Table 1) and plays a role in cooperation, parent-offspring identification and inbreeding avoidance via two different phenotype matching mechanisms: self reference or familial imprinting. Third, MHC signals are used to determine the genetic compatibility of a potential mate and can result in the production of heterozygous offspring. In some animals, mate choice for MHC compatibility is so finely tuned that they can optimize the degree of MHC heterozygosity in their offspring. Fourth, information regarding MHC genotype can be signaled indirectly through correlated characters (Table 2) and a recent study demonstrated that, at least within one species, MHC signaling itself may be condition-dependent and therefore a signal of individual quality. Taken together, these studies suggest that MHC-mediated signaling is conserved across vertebrates, but takes on unique functions depending on the life-history of a given species.

Appreciating the distinction between both modes of phenotype matching (self reference and familial imprinting) is paramount in understanding the role MHC-mediated signaling plays in social communication. Though substantial overlap in functionality exists between these phenotype matching systems, there are tradeoffs. Self reference systems facilitate mating preferences that generate offspring with an immunological advantage by allowing the assessment of genetic compatibility. Familial imprinting systems of phenotype matching facilitate the identification of siblings, half-siblings and cousins; in species where either cooperative behavior or avoiding inbreeding is important (e.g., communal nesting species or species that live in high-density populations), a familial imprinting system provides an advantage over a self reference system because self/nonself discrimination is not required to increase indirect fitness. That these two systems are differentially utilized by different groups of vertebrates highlights the highly context-dependent nature of social signaling. It is important to note, however, that phenotype matching mechanisms have been described in a relatively small number of species (Table 1) and more studies are needed.

The remarkable fact that a single genetic system controls major components of both immune recognition and social recognition begs the question of which recognition system constituted the primordial function of MHC genes. The convergent evolution of similar peptide binding properties of MHC, VSN and OSN receptor molecules provides the molecular basis by which MHC genotype influences both immune and social recognition; it also implies that these distinct receptor families have responded to selective pressures that required information regarding MHC genotype (bound peptides) be associated with discriminatory sensory systems. Finally, the ubiquitous presence of various modes of self versus nonself discrimination across all three domains of life, coupled with the derived nature of the adaptive immune system in vertebrates, further suggests that MHC-mediated social signaling evolved for the purpose of discrimination between conspecifics and could represent the ancestral state. Tracing the function of MHC molecules across vertebrate evolution holds the greatest promise of resolving the relative importance of immune versus social communication in MHC evolution.

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