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Female choice and the MHC

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In animals, it is the female that typically selects a mating partner. This decision can occur before, during and after copulation. Here, recent evidence for the involvement of genes within the *MHC* in female choice is reviewed and the roles of MHC I and II antigens, various types of chemoreceptors, as well as *MHC*-encoded transcription factors, in securing an optimal genetic constitution of the offspring are discussed. Some particularly interesting and as yet unanswered questions are raised and some experiments that could provide deeper insight into the molecular mechanisms underlying female choice are suggested.

Introduction

Following the 1976 discovery by Yamazaki and colleagues that *MHC*-dependent, individual-specific odours influence sexual selection in mice [1], several studies in vertebrates, including humans, have revealed that highly polymorphic genes within the *MHC* participate in mate choice [2]. Therefore, besides their crucial function in adaptive and innate immune responses, the products of *MHC* genes also have a role in behavioural contexts [3,4], as well as in reproduction. A detailed insight into the structure–function relationships of many molecules involved in immune reactions has already been achieved, however, only limited knowledge regarding the chemical nature of *MHC*-dependent odours, their recognition by chemoreceptors and how ligand–receptor interactions lead to neuronal responses and finally to *MHC*-influenced behaviours has been obtained. This deficit is probably a result of the complexity of the problem, which can be tackled only by a multi-disciplinary approach, involving, for example, studies of behaviour, genetics, immunology, neurobiology, chemistry and structural biology.

The connection between the *MHC* and mate selection is presumably a consequence of the crucial importance of the *MHC* in immune responses, in which two classes of genotypes might be favoured: heterozygotes and rare alleles [2,5–7]. *MHC* heterozygotes have an advantage over homozygotes because more peptides can be presented to T cells and rare *MHC* alleles might prevent pathogen evasion of host immune responses or exhibit a lack of association with autoimmunity, which has been shown by studies in a few vertebrate species, including humans [8–10]. These two mechanisms could select for *MHC* diversity but both would also lead to the evolution of preferences for mates that are *MHC* heterozygous and *MHC* distinct [5]. The avoidance of inbreeding is an added benefit that can be a consequence of choosing an *MHC*-heterozygous mating partner. There is also evidence that *MHC* heterozygosity can aid in the recognition of kin [5]. For example, in the males of many mammalian species, this leads to the avoidance of mating with their own mother [4]. Theoretical considerations indicate that, for a given individual, an optimal number of *MHC* alleles at an intermediate level of *MHC* diversity exists [11]. Recent experimental evidence supports this prediction [8,9].

In Darwin's view, sexual selection was restricted to the precopulatory phase, during which 'the females…select the more agreeable partners' [12]. However, within the last three decades, has come the realization that female choice (see Glossary) operates during and after copulation as well, and it influences the behaviour, anatomy and physiology of both sexes. Evidence from diverse animal groups also indicates that females use extra-pair matings to enhance the heterozygosity and fitness of

Glossary

Cryptic female choice: Possibility that sexual selection might involve hidden female effects that impact on the success of males in fertilizing ova.
Female choice: Selection of a mating partner by a female. This can occur before, during and after copulation.
Haplotype: Combination of alleles at several loci on a single chromosome of a given individual. For example, for a gene with alleles W and w that is linked to another locus with alleles V and v, possible haplotypes are WV, Wv, wV and wv.
Imprinting disorder: At an imprinted gene, only one of the two alleles is transcriptionally active owing to methylation of the other allele. In imprinting disorders, this methylation status is altered, such as in Beckwith-Wiedemann or Angelman syndromes.
Linkage disequilibrium: The non-random association of alleles at adjacent loci. It is a hallmark of many HLA haplotypes.
Main olfactory epithelium (MOE): Located in the distal part of the nasal cavity, it is the site in which odorant receptor genes are primarily expressed. Olfactory sensory neurons within the MOE project to distinct glomeruli in the main olfactory bulb.
Monoallelic expression: Expression of only one of the possible two alleles of a gene within a given cell. In the case of odorant receptors, only one of hundreds of *OR* loci and one of the two alleles of this gene are expressed within an olfactory sensory neuron.
Odourtype: Strain-specific urinary odour of an animal (usually rodents are being referred to).
Positive selection: A process in which more non-synonymous (amino acid-changing) than synonymous substitutions have been retained. It is observed when non-synonymous substitutions in a gene are selectively advantageous, for example, by increasing the fitness of a species.
Sperm competition: Although usually defined as competition between spermatozoa of two or more males to fertilize a given set of oocytes, it can also occur between sperm within one ejaculate.
Vomeronasal organ (VNO): Second olfactory system in many vertebrates. In the mouse, it consists of two blind-ended tubes in the proximal part of the nasal cavity. It is organized within two zones that exhibit differential expression of the two types of vomeronasal receptor genes, *V1R* and *V2R*. The neurons project to distinct glomeruli in the accessory olfactory bulb.

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their offspring [13,14]. Sperm competition, originally defined as the competition between spermatozoa of two or more males to fertilize a given set of oocytes, might also be connected with female choice [13–15].

We have only begun recently to understand some of the mechanisms that influence mate choice in vertebrates. This has been aided greatly by the molecular identification of odorant receptors (ORs) by Buck and Axel in 1991 [16] and the subsequent description of chemoreceptors (the vomeronasal receptors V1R and V2R) expressed within the vomeronasal organ (VNO) (reviewed in Ref. [17]). In addition, these different types of chemoreceptors not only fulfil their role of recognizing distinct ligands within the nose (an important process in the initial encounter of potential partners) but are also used by spermatozoa as guidance cues [18,19]. Possibly, their expression on spermatozoa enables a female to influence sperm motility through the establishment of chemical gradients within her reproductive tract. This indicates that postcopulatory female choice might involve gene products that take part in precopulatory mating decisions as well.

This review will focus on the evidence for the involvement of polymorphic *MHC* genes and chemoreceptors [17] in the various stages of female choice. The role of *MHC*-encoded transcription factors (TFs) that might influence prezygotic control within the fertilized oocyte will then be discussed, and analogies to mechanisms that participate in mate choice in fungi, currently one of the genetically best characterized systems [20], will be pointed out.

Precopulatory sexual selection

Circumstantial evidence for an influence of *MHC* alleles on precopulatory female choice has been observed in nearly all classes of vertebrates. However, evidence for a relationship among various receptors within the main olfactory epithelium (MOE) or the VNO (Box 1), the *MHC* and reproductive behaviours has been obtained only in a limited number of species, particularly rodents [21,22] (for a comprehensive review of earlier work, including that carried out with rats, see Ref. [2]). The involvement of *MHC I* genes in the generation of strain-specific urinary odours ('odourtypes') in mice is supported by several independent findings:

(i) Olfaction enables trained mice to distinguish single point mutations in the *MHC I* gene *H2-K* [2];

(ii) β_2-microglobulin (β_2m)-deficient mice have greatly reduced numbers of all molecules that associate with β_2m, such as MHC I heavy chains, and possess a different odour from that of non-mutant animals [23];

(iii) The natural *MHC I* mutant strains *bm1* and *bm3*, whose H2-K molecules differ from each other by only five amino acids that affect peptide binding, possess distinct odourtypes and can be distinguished by untrained mice [24];

(iv) Peptides derived from MHC I molecules function as sensory stimuli for vomeronasal neurons harbouring V2R [21]; and finally,

(v) In the mouse and human genomes, a large cluster of *OR* genes is tightly linked to the *MHC* [25,26]. This fact, together with the high degree of linkage disequilibrium between the two types of loci in humans (reviewed in Ref. [26]), suggests the existence of a functional connection between the *MHC* and *MHC*-linked *OR* loci [27].

Several attempts had already been made to identify the molecules involved in chemosignal-based communication but it is the study by Leinders-Zufall and colleagues [21] that shows unequivocally the influence of specific peptides that can be bound specifically by MHC I antigens on the generation of odourtypes. These peptides must meet precise structural requirements to be effective, that is, they have to retain those amino acids that act as anchors when presented by MHC I molecules to activate the same VNO neurons. Peptides derived from H2-D and H2-K antigens, even from different haplotypes, led to similar numbers of responding cells in VNO sections but they activated distinct V2R neurons. The authors also found that peptides could induce the termination of a pregnancy (Bruce effect). This was observed only when the peptides were derived from MHC molecules of a haplotype different from that of the mating male. Therefore, the VNO has a role in interacting with peptides derived from MHC I antigens. Because *V2R* expression requires the simultaneous presence of a particular group of MHC I molecules (M1 and M10) in V2R-bearing neurons [4,17], it might even be that these molecules, and not V2R, are responsible for peptide recognition by the respective neurons.

Box 1. Odorant and vomeronasal receptors

There are three different types of chemosensory receptors within the nasal cavity of many vertebrates (reviewed in Ref. [17]): OR (often also termed olfactory receptors) and the two vomeronasal receptors (V1R and V2R). Whereas *OR* genes are expressed in a monoallelic fashion by olfactory neurons within the MOE, V1R and V2R are found on two types of neurons that are located in different zones of the VNO [64]. OR are typically concerned with the recognition of environmental odours, whereas V1R and probably also V2R detect substances that are important in social and reproductive behavioural contexts ('pheromones'; note, however, that snakes can smell food odours with their VNO) [4]. OR, V1R and V2R are seven-transmembrane G-protein-coupled receptors that belong to distinct superfamilies. In contrast to the OR and V1R superfamilies, all V2R proteins contain a large N-terminal, extracellular domain.

The number of functional *OR* genes differs greatly between mammalian species [17]. For example, the mouse and dog genomes contain ~1400 *OR* genes, of which ~20% seem to be pseudogenes, whereas humans have ~900 *OR* loci, although only about half are potentially functional. The OR repertoire size is further increased by polymorphisms [49]. In humans, *OR* genes occur on nearly all chromosomes, usually as clusters [e.g. the large *OR* gene cluster linked to the human *MHC* is divided into two sub-clusters with a total of 34 members (Figure 1)].

In case of the genes expressed within the VNO, mice possess nearly 200, although dogs and humans have only 8 or 5 potentially functional *V1R* genes, respectively [65], and although at least 150 potentially functional mouse *V2R* genes have been identified, there is, however, not a single functional *V2R* gene in humans, dogs, cows and goats. For one of the few human *V1R* genes with an open reading frame, expression within the MOE has been demonstrated, so that its participation in olfaction seems possible (reviewed in Ref. [17]). Given the vestigial nature of the VNO, the greatly reduced number of potentially functional *V1R* genes and only *V2R* pseudogenes in primates, it is the MOE that has to be considered in connection with human olfaction. *V1R* and *V2R* pseudogenization also concurs with the loss of a whole set of non-classical *MHC I* genes that are expressed exclusively within those neurons of the mouse VNO that harbour V2R (reviewed in Refs [4,17]).

Further experiments must address the identity of the V2R that resides on a responding neuron and determines their degree of peptide-specificity, their mode of peptide binding and how they influence behavioural and neuroendocrine responses. In particular, the way in which information on the identity of an MHC I antigen is transferred by a released peptide to a V2R molecule needs clarification because there appears to be a fundamental difference in comparison to the recognition modes of MHC I or II molecules by peptide-restricted ligands involved in cellular immunity, such as T-cell receptors. X-ray crystallographic studies will probably be needed to answer many of the questions that we have raised.

VNO involvement does not preclude a role for the MOE in recognition of *MHC*-derived odours [28], however, and, indeed, urine from male mice differing in the *H2-K*^{b}, *K*^{bm1} or *K*^{bm8} alleles leads to activation of distinct glomeruli within the main olfactory bulb of female mice [22]. This is an anatomical structure that receives input from the MOE but not from the VNO. The urinary molecules responsible for this effect were not characterized, although the authors speculate that peptides bound to MHC molecules or their breakdown products might be responsible. This could be further investigated by using an experimental approach similar to that of Leinders-Zufall *et al.* [21], that is, by exposing the animals either to urine with additions of defined peptides or to the purified peptides themselves. As with complexes of V2R and MHC I antigens, an in-depth understanding of OR–ligand interactions will probably rely on structural studies of an OR with its cognate ligand. However, given that OR and V2R are seven-transmembrane G-protein-coupled receptors, this must be regarded as a difficult task because, to date, the structure of only one seven-transmembrane G-protein-coupled receptor, rhodopsin, has been determined [29].

Genes outside the *MHC* are responsible for specifying ~50% of an odourtype of a mouse. Polymorphic major urinary proteins (MUPs), which are excreted in large quantities in urine, contribute to these odourtypes and MUPs assure that bound, low molecular weight volatiles are released slowly from scent marks. In those few species (hamster, mouse and rat) in which MUPs and associated compounds have been investigated, it appears that the VNO is mainly responsible for their detection [4]. However, our current knowledge does not permit clear-cut demarcations of function for the MOE and OR on one hand, and the VNO and V1R and V2R on the other hand [4] (Box 1). Therefore, an interesting question remains: to what extent can the MOE take over functions of the VNO (and vice versa)? Clearly, the absence of a VNO in humans cannot be taken as evidence that all vomeronasal functions of mice have been lost in our species.

Great advances have also been made recently in *MHC*-associated mating behaviours in fish. Gravid female sticklebacks (*Gasterosteus aculeatus*) assess the odour of potential mates to deduce their *MHC IIB* allele number [30]. They use this information in combination with 'knowledge' about their own *MHC* polymorphism ('self-reference') for optimal complementation of their own set of alleles [31]. Furthermore, the addition of synthetic peptides selected from stickleback protein sequences can predictably modify the response of females that are exposed to water containing male odours. For a mating pair with a suboptimal number of *MHC* alleles, added peptides increase the attractiveness of male water, whereas it is decreased when the pair already possess a superoptimal *MHC* allele combination [32]. Similar to the situation in mice [21], the peptide C-terminus (also a presumed anchor residue for MHC I molecules of sticklebacks) is important for the perception of the peptides by females [32]. In conclusion, two independent studies, with distinct designs and in different classes of vertebrates, now implicate MHC-bound peptides as chemosignals in *MHC*-associated precopulatory reproductive behaviours [21,32].

In comparison to these experiments in fish and rodents, our knowledge of *MHC*-based precopulatory female choice in reptiles, birds and primates, including humans, is at best fragmentary. Female sand lizards (*Lacerta agilis*) prefer odours of males with a different *MHC* from their own [33] and the situation was similar in a free-living population of Savannah sparrows (*Passerculus sandwichensis*) [34], however, females of the great snipe (*Gallinago media*) prefer males with particular *MHC IIβ* alleles [35]. The analysis of mate preferences in different human populations is made particularly complicated because religious, ethnic and social aspects have a role in reproductive behaviours. This results, for example, in a considerable degree of consanguinity in many parts of the world [36], and conflicting data were obtained with regard to an influence of the *MHC* on mate selection [2]. An involvement of *MHC* alleles was either observed, as in Hutterites, an isolated sect in North America, or not found, as in South Amerindians. However, the human *MHC* (the *HLA* complex) also influences body odours and olfactory preferences that are not restricted to women but that can also be observed in men. Comparable studies in Hutterites revealed that females exhibit a preference for paternally inherited *HLA* alleles [37]. These results have been critically discussed [5,38] because they differ from most previous studies in humans and animals. Nevertheless, the preference for basic ingredients of perfumes also correlates with *HLA* type [39].

Postcopulatory sexual selection before oocyte fertilization

As a costly investment into an embryo with potentially suboptimal genetic and immunological properties should be avoided by a female, it would seem to make sense for her to scrutinize the suitability of the genetic contribution of the male before zygote formation. This could occur by cryptic female choice in connection with sperm competition, as well as by prezygotic female choice during and after fertilization of the oocyte (see the next section). The term 'cryptic female choice' refers to the possibility that sexual selection might involve hidden female effects that impact on the success of males in fertilizing ova [40]. It is particularly pronounced in those species in which females mate with several partners in rapid succession, enabling the female to influence the fate of individual spermatozoa, which are in addition subject to sperm competition [13–15], by various means. In mammals [14], these mechanisms of postcopulatory female choice include low

vaginal pH (reduction of sperm survival), sperm capacitation and hyperactivation of swimming movements induced by oviductal stimuli, including chemical cues, and oviductal length, which is greatest in those species in which sperm competition is most intense [14,15].

Sperm competition can, in principle, also take place between spermatozoa within one ejaculate. A striking example is presented by male mice that are heterozygous for wild type and *t*-complex haplotypes. The *t*-complex has so far been found only in mice and is a variant region of the *MHC*-carrying chromosome that exists as a naturally occurring polymorphism [41]. Spermatozoa that harbour the wild type (+) allele usually have a greatly diminished chance to fertilize an oocyte (<50% down to 1%) in comparison to t-bearing spermatozoa. However, despite this advantage, *t* haplotypes do not expand in wild mouse populations, probably owing to a fitness advantage that +/+ males have over +/*t* males in maintaining their territory, leading to female choice of +/+ males [42]. These results provide a convincing demonstration that pre- and post-copulatory sexual selection mechanisms can be intertwined inextricably, and that *MHC* alleles might be inherited in non-Mendelian fashion owing to sperm competition.

Postcopulatory sexual selection has been extensively reviewed recently, mainly from an evolutionary and ecological perspective [43]. The authors also considered the involvement of the *MHC* and pointed out that MHC antigens might be expressed on the surface of spermatozoa to enable female recognition of the alleles of the sperm. This might seem an attractive possibility, however, the situation is more complex: despite some controversy, several groups agree that neither MHC I nor II molecules are expressed on mature spermatozoa [44]. The sperm receptor selection (SRS) hypothesis [45] could offer a solution to this dilemma (Box 2). It argues that polymorphic ligands within the female genital tract (e.g. soluble MHC molecules, their fragments or MHC-bound peptides after dissociation) might only be recognized by a spermatozoon if the ligands appear as 'non-self'. This implies that the female has the chance for selection between spermatozoa from different males and between spermatozoa within the ejaculate from a single male as well. However, despite the preference that genetically distinct sperm will be given, it is obvious that an interaction between gametes with identical *MHC* haplotypes can take place, as shown by the existence of *MHC*-homozygous individuals. Support for the SRS hypothesis (Box 2) might come from experiments in which the influence of self- or non-self-HLA class I molecules, their fragments or HLA-bound peptides on the motility of spermatozoa from HLA-typed men were investigated.

The monoallelic *OR* expression found within olfactory neurons is not observed in male germ cells (Box 2). At least ~50 distinct *OR* genes are expressed in the mammalian testis [46–48]. All spermatozoa within an average human ejaculate (~$2–300\times10^6$) might thus exhibit different ligand specificities if only five of these distinct *OR* genes, but in random combination, were expressed on a given cell. OR polymorphisms [49] are likely to increase the total number of different ORs on spermatozoa even further, possibly extending the repertoire of the recognized ligands. Differential expression of chemoreceptors on spermatozoa might thus promote both cryptic female choice and sperm competition.

Box 2. SRS hypothesis

Polymorphic MHC I or II antigens are neither expressed on spermatozoa nor on human oocytes [44]. However, to enable female choice, spermatozoa should ideally signal which *MHC* haplotype they carry and they should also advertise all of the 'self' of the male, allowing the female to assess whether this optimally complements her own 'self', thus permitting fertilization. The SRS hypothesis [45] suggests that this is accomplished by a process that bears some resemblance to negative T-cell selection within the thymus:

(i) A large variety of male self-molecules expressed by cells of the testicular seminiferous epithelium could interact with chemoreceptors (OR, possibly also V1R or even V2R, depending on the vertebrate species), leading to the exclusion of self-reactive receptors from sperm expression by an as yet unknown mechanism.

(ii) Consequently, only receptors that lack self-reactivity will appear on the surface of spermatozoa.

(iii) Spermatozoa carrying these receptors will respond optimally to chemical stimuli produced by the female if these are different ('non-self') from those that the receptors had been exposed to within the testes.

The SRS hypothesis predicts that:

(i) Soluble self-MHC antigens will not be recognized by spermatozoa (clearly a necessity because of the presence of these proteins within the seminal fluid).

(ii) Sperm competition might find a molecular explanation through the mutual recognition of seminal fluid components (including soluble MHC antigens) by spermatozoa from different males. This could influence directional spermatozoal motility and thus their fertilizing potential.

(iii) Fertilization of oocytes by genetically different sperm will be favoured, whereas fertilization by genetically identical or similar sperm will be discouraged, thereby providing a prerequisite for the occurrence of cryptic female choice.

The SRS hypothesis is supported by several findings [45], including the fact that a given *OR* gene is expressed only within a fraction (~30–90%) of spermatogenic cells (mainly spermatids). This has been observed in humans [46,57], mice [19,66,67], rats [66,68] and dogs [47]. In line with this, the product of a particular *OR* gene is expressed on a variable number (~5–40%) of spermatozoa within a given ejaculate in humans, mice and dogs [18,19,69]. In addition, sperm-expressed human and mouse OR are functional, affect sperm motility after interaction with specific ligands and exhibit the same specificity as in the MOE. Furthermore, *V1R* genes might also show testicular expression because transcripts are found in ~30% of mouse spermatids [67].

Postcopulatory sexual selection during and after oocyte fertilization

The interaction of male gametes with an oocyte depends on co-evolving proteins that are present on both types of cells [50]. In vertebrates, none of the proteins involved are known to be *MHC*-encoded, although, similar to MHC antigens, several are highly polymorphic. This additional barrier to fertilization might participate in cryptic female choice and act on the few mammalian spermatozoa (in humans, maximally a few hundred) that reach the vicinity of an unfertilized oocyte. These considerations are of great interest in the context of reproductive medicine, in which 3×10^5 spermatozoa are typically mixed with an oocyte during *in vitro* fertilizations (IVF). Despite this deviation from the natural setting, in ~50% of cases IVF leads to the formation of a zygote. Another assisted

reproductive technology, intracytoplasmic sperm injection (ICSI), yields a zygote in ~60% of fertilizations. In each of these two techniques, ~25% of the fertilizations develop into a pregnancy, and a child is born in about half of these pregnancies (www.deutsches-ivf-register.de). Interestingly, IVF and ICSI have been associated with a slightly increased risk of congenital malformations in the children [51], also including imprinting disorders, such as Beckwith-Wiedemann syndrome and Angelman syndrome [52]. However, an understanding of the underlying mechanisms is hampered by our current ignorance of many molecular details.

IVF experiments with cells from male and female mice that differ at the *MHC* indicate female choice for distinct sperm *MHC* haplotypes. Furthermore, it is the *MHC* type of the fertilizing spermatozoon that influences the outcome of the second meiotic division in the oocyte (this occurs in many vertebrates after the sperm has penetrated the vitelline membrane of the egg) [53]. In addition, in an experimental setting in which *MHC*-heterozygous and -homozygous embryos should have been produced at the same rate, females carried more *MHC*-heterozygous embryos when the parents were infected with hepatitis virus, suggesting that parental infection affects the degree of MHC heterozygosity in the offspring [54]. *MHC*-influenced sexual selection within the oocyte must therefore be regarded as a distinct possibility, at least in mice. Some invertebrates and plants provide compelling examples for prezygotic female choice after fertilization: for instance, in the comb jelly *Beroë ovata*, an oocyte is usually fertilized by more than one sperm. Following the entry of the sperm nuclei into the oocyte cytoplasm, the female pronucleus evaluates the male pronuclei one after the other during an inspection-like process before fusion occurs with one of them (http://biodev.obs-vlfr.fr/recherche/biomarcell/ctenophores/beroe.htm).

Could genes within the *MHC*, other than class I or class II loci, be involved in vertebrate female choice (Box 3)? Among the genes that have an indispensable role in vertebrate embryogenesis, *POU5F1* (POU domain, class 5, transcription factor 1) exhibits an evolutionarily conserved location within the *MHC* [26,55] (Figure 1). It encodes a TF that is crucial to maintain totipotency in embryonic stem cells and, together with other TFs, directs

Box 3. Prezygotic female choice within the fertilized oocyte

In certain fungi, a double barrier guarantees that only unrelated organisms, with two different mating types, successfully complete sexual reproduction. For example, in the inkcap (*Coprinus cinereus*), mate selection is governed by two different sets of genes that together determine ~12 000 mating types. Compatible mates possess distinct alleles at both groups of genes. The first consists of a sophisticated system of multiple clustered genes for pheromones (lipid-modified peptides) and pheromone receptors (seven-transmembrane G-protein-coupled receptors), whereas the second is formed by linked loci that encode a TF heterodimer. To complete the life cycle, both groups of genes have to act in a sequential, predetermined fashion. It might be that fusion between vegetative hyphal cells of the two mating partners occurs, but if a TF heterodimer cannot form, then a new, diploid organism will not be generated [20].

The constituents of both systems are highly polymorphic and the first clearly shows remarkable parallels to genetic systems that contribute to mate choice in vertebrates [MHC antigens or peptides and OR or VNO receptors (Box 1)]. The principal similarity between the second fungal mating type system and vertebrate TF involved in sex determination has been noted before but its significance is unknown [20]. If *MHC* genes are also involved in mate choice decisions through interacting TF, then several candidate loci would have to be considered. Apart from *POU5F1*, which has a crucial role in embryonic development, the *MHC* contains further genes for TF (Figure 1), for example, *TCF19* and *GTF2H4* (closely linked to *POU5F1* in the class I region), *PBX2* (class III region), *BRD2* (class II region), *RXRB* (extended class II region) or *PHF1* (at the centromeric boundary of the extended *MHC*), as well as many zinc-finger loci [26]. *MHC*-linkage of several of these genes is highly conserved [55] (Figure 1) and some are polymorphic. For example, polymorphism of the human *POU5F1* locus yields two mRNAs with different lengths [70]. The resulting proteins are expected to differ substantially at their N-termini and probably possess an altered capability to interact with other TF that influence the transcriptional specificity of the POU5F1 protein [56]. It remains to be seen whether a connection to sexual selection mechanisms will be found for any of these genes, and whether both gametes contribute to the initial transcriptional activity within the oocyte before formation of the zygote (compare Refs [20,58,59]).

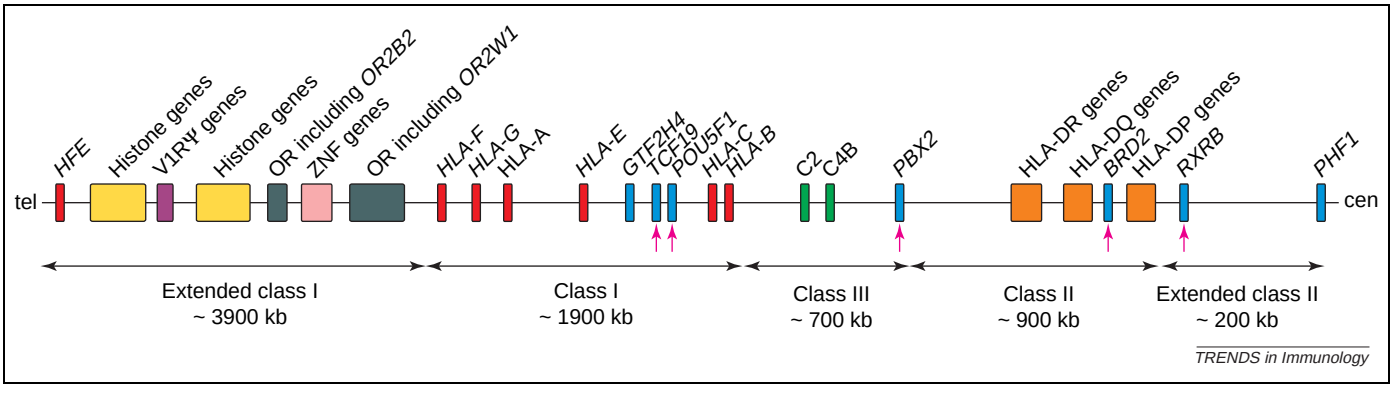

Figure 1. Map of the extended human MHC. The map (not to scale) shows selected genes and gene clusters of the extended MHC (xMHC) from telomere (tel, left) to centromere (cen, right) on the short arm of human chromosome 6. The total number of genes encoded within the xMHC is 578 [26]. The five subregions making up the xMHC span ~7600 kilobasepairs (Kb) and are indicated by arrows below the map, with their approximate lengths. The following types of genes are mentioned within the review: class I genes (red), class II genes (orange), OR gene clusters (dark green), V1R pseudogene cluster (violet), zinc finger genes (pink, only one of the several locations of ZNF loci is shown) and TF genes (blue). The red arrows indicate those TF genes whose location within the xMHC is conserved evolutionarily from fish to mammals. The following genes with their symbols are depicted: *HFE*, hemochromatosis; *OR2B2*, olfactory receptor, family 2, subfamily B, member 2; *OR2W1*, olfactory receptor, family 2, subfamily W, member 1; *GTF2H4*, general transcription factor IIH, polypeptide 4; *POU5F1*, Pou domain, class 5, transcription factor 1; *TCF19*, transcription factor 19; *C2*, complement component 2; *C4B*, complement component 4B; *PBX2*, pre-B-cell leukemia transcription factor 2; *BRD2*, bromodomain-containing protein 2; *RXRB*, retinoid X receptor, β; *PHF1*, PHD finger protein 1. The *POU5F1* gene is also known as *Oct4* in the mouse. Further details can be found in recently published reviews [26,55].

the establishment of the first three lineages in mammalian embryos [56]. We have argued previously that *POU5F1* might contribute to cryptic female choice [57] and the recent observation that an individual mouse spermatozoon can deliver ~18 000 male transcripts [58], as well as a variety of TF [59], to the oocyte could also be relevant in this context.

Concluding remarks

Clearly, MHC molecules have a crucial role within the immune system, however, there is also growing evidence for a role in the refinement and plasticity of neuronal connections [3] and during distinct phases of reproduction, as summarized here. Many genes that fulfil essential functions within these three systems are in tight linkage with the *MHC* [26,55] and are subject to positive selection. - For example, two *HLA*-linked *OR* genes, *OR2W1* (olfactory receptor, family 2, subfamily W, member 1) and *OR2B2* (olfactory receptor family 2, subfamily B, member 2) (Figure 1), which are also testis-expressed [48], are among the top 0.3% of human genes that experience selection in favour of genetic variants [60]. The *MHC* as a functional unit should thus not be thought of as containing only the class I, II and III regions because a much larger chromosomal segment (in humans, ~7600 kilobasepairs, Figure 1) is kept together by linkage disequilibrium [26]. Consequently, this region has been designated the 'immuno–olfactory supercomplex' [27] or the 'extended *MHC*' [26].

In humans, there is still uncertainty as to whether the sharing of MHC alleles between the partners influences the occurrence of certain forms of human sterility and recurrent spontaneous abortions [61]. However, a role for several trophoblastic *HLA-C* alleles, together with maternal alleles of killer Ig-like receptors, in influencing reproductive success is made probable by studies of spontaneous abortions [62] and of pre-eclampsia, another serious pregnancy complication [63]. In addition to this recent progress in the field of human reproduction, it is obvious that the involvement of various chemoreceptors in detecting *MHC*-determined chemosensory cues within the nasal cavity [21,22,28], as well as the presence of functional ORs on spermatozoa [18,19], has opened an entirely new area of interdisciplinary research.

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