INTRODUCTION TO MHC GENES

The genes of the major histocompatibility complex (MHC) are recognized as an essential component of the vertebrate adaptive immune system. The major histocompatibility complex is a system of genes evolved in vertebrates as a response to increase the protection against parasites. The role of MHC molecules is to present parasite-derived peptides to T cells, which subsequently initiate a specific immune response. These molecules consist of three modules that arose independently: the membrane-anchoring module (MAM), the immunoglobulin-like module (ILM) and the peptide-binding module (PBM) (Klein & O’hUigin 1993, Klein et al. 1997). Two groups of MHC molecules – class I and class II have been recognized and described in teleost fish (as well as in other jawed vertebrates). MHC class I molecules bind peptides derived from intracellular pathogens (viruses and bacteria), whilst MHC class II molecules bind peptides derived from extracellular pathogens (especially macroparasites). In teleost fish, MHC class I and class II represent two independent linkage groups. Class I and class II MHC molecules appear to have the same structure and the encoding genes have basically the same exon-intron organization in fishes, amphibians, “reptiles”, birds and mammals (Klein et al. 1997). Flajnik & Kasahara (2010) proposed that major features of MHC-based adaptive immune system arose early in “big-band” and have been modified only slightly over evolutionary time. Therefore, this system displays conserved and plastic features, the former allows for a general function of the system and the latter permits rapid changes as a response to parasites.

The binding between MHC molecule and foreign peptide is realized by a small number of amino acid residues of the peptide-binding region (PBR) specified by exons 2 and 3 in the class I A gene and exon 2 in class II A and II B genes. The PBMs of class I and class II molecules accommodate peptides of different lengths and constitutions. Each PBM is capable to bind a large array of peptides that share amino acid residues at a few positions. The bound peptides derived from parasites with MHC molecules are recognized by specific receptors on T lymphocytes.

In fish, the first studies describing the sequence variability of MHC genes and the exon-intron organization of MHC genes were published for cyprinids (e.g. Ono et al. 1992, Okamura et al. 1993, Sültmann et al. 1993, 1994), cichlids (e.g. Klein et al. 1993, Ono et al. 1993a, b), and salmonids (e.g. Juul-Madsen et al. 1992, Grimholt et al. 1994). From the 1990’s, fish MHC genes have attracted more attention in the fields of molecular biology, evolutionary ecology, and behavioral studies, which have involved increasing numbers of fish species being screened for their MHC profiles. In 2008, Wegner et al. identified 3559 MHC class I and class II sequences in 137 species covering most major orders of teleosts, and the three families mentioned above exhibited the highest number of species and the highest MHC sequence diversity.
MHC IIB GENES IN CYPRINID FISH

Concerning cyprinids, MHC genes were first described in common carp (Cyprinus carpio). The first two DAB alleles of MHC IIB genes (i.e. Cyca-DAB1 and Cyca-DAB2, respectively) were identified by Ono et al. (1993b) and recognized as the alleles of a single locus (later designated as the alleles of the DAB1 lineage or DAB1-like alleles). Van Erp et al. (1996) proposed that these alleles are the result of recent gene duplication and identified two new DAB alleles (Cyca-DAB3 and Cyca-DAB4) as the alleles of another locus, later designated as the alleles of the DAB3 lineage or DAB3-like alleles. Studies performed on different cyprinid species using Sanger sequencing or next generation sequencing techniques (NGS) showed that the specimens of evolutionary diploid cyprinid species possess the alleles of one locus (i.e. either DAB1-like or DAB3-like) or the alleles of both loci (i.e. both DAB1-like and DAB3-like) which was revealed by the analyses of genomic and expressed DNA (Stet et al. 1997, Rakus et al. 2003, Ottová et al. 2007, Seiferova & Šimková 2011, Šimková et al. 2013a, b). A segregation study and phylogenetic analyses (Van Erp et al. 1996, Ottová et al. 2007, Seiferova & Šimková 2011, Šimková et al. 2013a) suggested that the DAB1 lineage and DAB3 lineage represent two pairs of closely related loci that segregated independently from each other. When comparing the expression profile among different cyprinid species, the number of MHC alleles at the individual level reveals the duplication within the DAB1 lineage in some cyprinid species (e.g. common carp by Rakus et al. 2009), the duplication within the DAB3 lineage in some cyprinid species (European chub (Squalius cephalus) by Seiferova & Šimková 2011), or the duplication of both lineages in other cyprinid species (the diploid form of three-spined stickleback (Gasterosteus aculeatus) by Seiferova & Šimková (2013b), South-west European nase (Parachondrostoma toxostoma) and common nase (Chondrostoma nasus) by Šmídková et al. (2013a)). Using Sanger sequencing, a usually lower number of DAB alleles per individual is reported in different cyprinid species (1-3 by Ottová et al. 2005, 2007, Šimková et al. 2013b). In contrast, using NGS, a higher number of DAB alleles per individual was found in other cyprinid species (1-6) and it was also shown that widely distributed cyprinids tend to express a higher number of DAB alleles at the individual level when compared to endemic cyprinid species with limited distribution (Šmídková et al. 2013a). However, even when using the classical sequencing method, an extraordinary diversity at the individual level (from 2 to 9 alleles) was reported in some non-cyprinids such as three-spined stickleback Gasterosteus aculeatus (Reusch et al. 2001, Wegner et al. 2003), suggesting even more extensive duplication within MHC IIB in some diploid fish species.

The aim of this study was to review the knowledge on origin and maintenance of MHC polymorphism in fish, to summarize the mechanisms contributing to the evolution of this polymorphism in fish, and to present the recent knowledge on MHC variability in hybrid systems, and diploid-polyploid fish complexes exhibiting asexual and sexual forms of reproduction.

ORIGIN AND MAINTENANCE OF MHC POLYMORPHISM IN FISH

The MHC alleles cluster into groups that are referred to as allelic lineages (e.g. the DAB1 lineage and DAB3 lineage in fish, see above). In the phylogenetic tree, the alleles of the same lineage from different cyprinid species cluster together and separate from the clade of the alleles of the other allelic lineage (e.g. for cyprinid species see Ottová et al. 2005). This indicates the trans-species character of MHC polymorphism, which is considered as evidence that the divergence of MHC lineages predicates specialization (Klein 1987).

Several mechanisms were proposed to explain the mechanisms maintaining such high MHC polymorphism. Recombination, gene conversion, and selection significantly contribute to the polymorphism of MHC genes. Recombination eliminates linkage disequilibrium between different combinations of mutations and combines beneficial mutations from different allelic lineages or eliminates detrimental mutations. Concerning fish, widespread intergeneric and intragenic recombination were identified in some species, e.g. three-spined stickleback (Reusch et al. 2004, Reusch & Langefors 2005). However, recombination does not seem to play an important role in maintaining the variation of MHC genes in all fish species, as demonstrated in the study of DAB genes of European chub by Seiferova & Šimková (2011).

The extensive polymorphism in MHC genes is especially pronounced in the codons encoding the peptide binding regions (PBR) of the MHC molecule (Hughes & Nei 1989). This is evidenced by a signal of positive selection classically detected by the significantly higher rate of non-synonymous nucleotide substitutions compared to synonymous substitutions (documented for fish, for example, in Ottová et al. 2005, Schaschl & Wegner 2006). As the PBR of fish have not yet been determined, their putative positions may be determined using the homology with human HLA genes (PBR of human HLA were published by Brown et al. 1993). However, the application of this method suggests differences in the positions of human and fish PBR (e.g. Miller & Withler 1996, Hedrick et al. 2001, Seiferova & Šimková 2011). Later, codon-based methods of estimating selection in a phylogenetic context and taking recombination into account were developed (REL and FEL methods, Kosakovsky Pond & Frost 2005, Kosakovsky Pond et al. 2005) and applied to detect the positively selected sites (PSS) in European chub (Seiferova & Šimková 2011). More
recently, the presence of selection is examined using the maximum likelihood method comparing different models with and without selection incorporated in order to test for the presence of sites under selection and to identify these sites (Yang et al. 2000, Yang 2007). In several cyprinid species, a codon-based analysis of selection was performed separately for the DAB alleles of two lineages, i.e. DAB1-like and DAB3-like alleles. Using this approach, Seifertová & Šimková (2011) showed a higher number of PSS in DAB3-like alleles compared to DAB1-like alleles, indicating stronger positive selection in DAB3 genes and suggesting a potential structural and functional difference between DAB1-like and DAB3-like genes. Ottová et al. (2005), investigating the selection pressures on exon 2 of both DAB lineages in a wide range of cyprinid species, proposed the secondary acquisition of the function of DAB1 genes after duplication as they did not identify selective pressure on the most basal branches of the DAB1 lineage in the cyprinid phylogenetic tree reconstructed using DAB1 and DAB3 sequences.

Parasite-mediated selection (balancing selection), and reproductive mechanisms based on mating preferences (Penn & Potts 1999) are the most often cited mechanisms maintaining extensive MHC polymorphism (see below for both mechanisms). However, in natural host populations, it is difficult to estimate the relative role of parasite-mediated selection in MHC polymorphism as the different mechanisms may operate together with other selective and neutral forces (Apanius et al. 1997, Spurgin & Richardson 2010). MHC diversity in natural populations is affected by neutral evolutionary processes, such as demography, genetic drift, and mutation (Bernatchez & Landry 2003). For example, Seifertová et al. (2016) used MHC and microsatellite data to show that the genetic structure of European chub populations across Europe reconstructed on the respective marker is similar, suggesting that neutral evolutionary processes like genetic drift may prevail over balancing selection (potentially mediated by parasites) in maintaining MHC polymorphism in wild fish populations. A similar study was performed for three-spined stickleback at the local level, in which Eizaguirre et al. (2011) showed that fish populations from lakes and rivers in Northern Germany possess different MHC profiles likely resulting from the combination of genetic drift and parasite-mediated selection.

PARASITE-MEDIATED SELECTION OF MHC GENES

As mentioned above, high MHC polymorphism is explained by two non-mutually exclusive hypotheses – parasite-mediated selection and sexual selection. Basically, two principal hypotheses have been proposed to explain how MHC polymorphism is driven by parasites (Hughes & Nei 1988, 1992, Klein 1991, Hughes et al. 1994, Hedrick 2002). First, the hypothesis of heterozygote advantage (overdominant selection) highlights the advantage of being a heterozygote at MHC loci as heterozygotes are able to recognize a wider range of antigens derived from parasites than homozygotes do. In fish, this hypothesis was empirically supported by several studies. For example, Arkusch et al. (2002) showed that MHC heterozygotes of winter-run chinook salmon (Oncorhynchus tshawytshcha) had higher survival rates than MHC homozygotes after exposure to infectious hematopoietic necrosis virus and outbred fish had a higher resistance than inbred fish after exposure to the myxozoan parasite Myxobolus cerebralis. A modification of the hypothesis of heterozygote advantage is the hypothesis of divergent allele advantage (Wakeland et al. 1990), according to which specimens with more divergent allelic combinations present a greater variety of antigens in their immune system (Lenz et al. 2009). However, within wild host populations, numerous pairs of associations between a single parasite and a host resistance allele contribute to the diversification of MHC genes, i.e. balancing selection (Apanius et al. 1997). Wegner et al. (2003) showed that populations of three-spined stickleback exposed to a wider range of parasites tended to be more diverse in MHC IIB genes. Thus, MHC diversity in wild species is more likely a result of many-to-many host gene/parasite coevolution (Wegner et al. 2003, Goüy de Bellocq et al. 2008). Second, the hypothesis of rare-allele advantage (frequency-dependent selection) is based on the prediction that the frequencies of MHC alleles continuously change with the frequencies of adapted and non-adapted parasites (Clarke & Kirby 1966). Thus, an MHC allele providing a better immune response to a parasite is, at the beginning, selected in a host population and subsequently increases in frequency. Associations between host MHC alleles and parasites fluctuate over time on the basis of coevolutionary arms races between hosts and parasites, which creates cycles of frequency-dependent selection. This means that the high frequency of a given MHC allele determining host resistance increases selection on a parasite to escape recognition by a host with this common MHC allele. Subsequently, such a parasite will spread in a host population and will impose selection against the common MHC host allele, which in turn imposes selection on the host to defend against the successful genotype of the parasite. The specific associations between an MHC haplotype or an MHC allele and a specific parasite were shown in several studies on fish. For example, Eizaguirre et al. (2011) demonstrated that the specific MHC haplotype was associated with a reduced load of Gyrodactylus species in river populations, whereas this haplotype was associated with an increasing Gyrodactylus load in lake populations.

However, Nowak et al. (1992) developed a theoretical model postulating an intermediate number of MHC alleles as the optimal MHC genetic spectrum for an individual. Following this model, the maximum possible number of...
MHC alleles is disadvantageous for an individual because it results in the presentation of more self-peptides with the subsequent elimination of self-reactive T-cells. In agreement with this hypothesis, Wegner et al. (2003) showed that at the individual level, an intermediate number of MHC IIB alleles was associated with minimal parasite load in three-spined stickleback.

Using human parasites, Klein & O’Huigin (1994) tried to delimit the categories of parasites with potential effects on MHC polymorphism. The first group is constituted by recent virulent parasites, which switch hosts with likely little effect on MHC, as the majority of non-synonymous PBR substitutions and MHC lineages are older than such parasites. The second group represents parasites coevolving with their hosts (i.e. with host immune systems); thus, these parasites were a potential source of selection pressure in the past. The last group represents well-adapted parasites normally not causing any measurable damage. The true nature of these parasites is revealed only when the host becomes immunologically compromised. Klein & O’Huigin (1994) proposed that only parasites of the second and third categories are responsible for the evolution of MHC polymorphism. However, Penn & Potts (1999) hypothesized that MHC may even act and to protect the host against rapidly evolving parasites (through heterozygote advantage, see below). Some studies performed in fish suggest that species rich, host-specific, and coevolving monogenean parasites represent the driving force maintaining the high diversity of MHC genes in cyprinids (Seifertová et al. 2006, Šimková et al. 2012, 2013). However, it should be taken into consideration that the load of these ectoparasites is also influenced by external factors and potentially by other host genes.

Host immunity develops throughout host’s life history. As immune response has a cost, it reduces another component of host fitness on the basis of the trade-off concept (i.e. if there are benefits in immune defense, other fitness components are reduced). In line with this prediction of life history theory, Šimková et al. (2006) hypothesized that high parasite diversity is the driving factor behind the diversification of MHC genes at species level and performed comparative analyses by investigating the potential associations between MHC variability and life history traits in cyprinid species. They showed that cyprinid species harboring high parasite species richness maintain high MHC genetic diversity, allowing them to decrease their natural mortality.

As MHC is one of the key determinants of adaptive immunity and immunity represents an important component of life history, a trade-off between investing in MHC and other components of life maintenance should be expected also within species. Kurtz et al. (2006) showed that an increasing activation of the immune system measured by MHC class II expression is correlated with the costs of higher levels of oxidative stress. Individual MHC class I and class IIB diversities were shown to be associated with male and female reproductive traits (Jäger et al. 2007). Males three-spined stickleback maintaining a high-quality nest carried an intermediate number of MHC IIB alleles. In addition, decreasing nest quality was associated with increasing parasite load under restricted food conditions. Interestingly, male breeding coloration, which is supposed to play an important role in mating selection (see below), was significantly correlated with an increasing number of MHC class I alleles but no association was found with MHC class IIB alleles. Concerning females, a conditional spawning strategy that depended on the presence of the specific MHC class IIB allele was detected. Females with abundant resources and possessing the specific allele, which probably allowed them to resist a common parasite, used a continuous spawning strategy (Jäger et al. 2007). Wegner et al. (2007) performed a selection experiment even demonstrating a trade-off between innate and adaptive immunity. More specifically, they showed that MHC class IIB expression is increased by approximately 50 % in three-spined stickleback lines selected for higher parasite load (i.e. low innate response). Even producing MHC signals is costly, therefore only replacatively active males produce such signals (Milinski et al. 2010) (see below).

**GOOD OR COMPLEMENTARY GENES: THE ROLE OF SEXUAL SELECTION IN THE EVOLUTION OF MHC POLYMORPHISM**

If parasites drive MHC polymorphism through one of the mechanisms, heterozygote advantage or rare-allele advantage, MHC-dependent mating preferences in some species are also selectively favored and these preferences further drive MHC polymorphism (Potts & Wakeland 1993, Penn & Potts 1999). However, MHC-dependent mating preferences also have the function to avoid kin matings and the deleterious consequences of inbreeding (i.e. high expression of recessive deleterious mutations and destruction of any heterozygote advantage), which is especially obvious in species at risk of inbreeding (as predicted by the inbreeding avoidance hypothesis).

Basically, MHC-disassortative mating preferences leading to heritable genetic quality work on the predictions of good genes or complementary genes (Neff & Pitcher 2005). Good gene models predict additive benefits of female choice; thus, some males exhibiting the more elaborated trait may represent superior partners for all females in a given population. These models are based on Hamilton & Zuk’s (1982) hypothesis predicting that a choosy female, after inspecting specific traits in males, selects her mate with the more elaborated trait, e.g. bright coloration, loud song, or vigorous movement. As the expression of such traits is costly for males, they must reflect good vigor and health status. In particular, such traits in the male indicate that the selected individual
carries the genes of resistance against the disease. The choosy female, by selecting a suitable mating partner through bright signals (e.g., long feathers, bright colors, loud songs and/or vigorous dances), is able to recognize good genes for her offspring. In contrast, the genetic benefits of female choice in the case of compatible genes is non-additive, and only a particular combination of male and female alleles is advantageous for offspring resistance to the disease. If sexual selection favoring compatible genes plays a role in mate recognition, females should smell out partners that provide complementary genes in order to produce offspring with an optimal number of different MHC genes. The majority of studies investigating MHC-linked mating preferences were performed in three-spine stickleback. Reusch et al. (2001) showed that reproductive females of three-spine stickleback from populations with high MHC diversity preferred the odor of males with a large number of MHC IIB alleles, but did not exhibit a preference for males with dissimilar genotypes when compared to their own genotypes. Aeschlimann et al. (2003) performed the same experiment with this fish species but from populations with low MHC diversity (corresponding to a natural population recently experiencing a bottleneck or to a population with a very low effective population size) and reanalyzed the data from the study by Reusch et al. (2001). They showed that in low population sizes, females strongly preferred males with dissimilar MHC alleles. The reevaluation of the data used in Reusch et al. (2001) showed that in the case of high population sizes, females exhibited a mating strategy to achieve an optimum number of complementary MHC IIB alleles for their offspring. Therefore, finally, a complex strategy of self-reference to optimize the MHC allele number during mate selection was proposed, i.e., the female knows her own alleles (self-reference) and knows the combinations of MHC alleles when choosing a male. In the Chinese rose bitterling (Rhodeus ocellatus), females preferred MHC dissimilar males. Using in vitro fertilization technique to avoid the role of maternal effect, Agbali et al. (2010) demonstrated that offspring rate of development and survival was linked to female preference; offspring produced with the preferred male survived much better than offspring produced with non-preferred males. In that study, male MHC dissimilarity was proposed to indicate genetic compatibility, with major fitness consequences (Agbali et al. 2010). A mating preference for MHC-dissimilar versus MHC-optimal compatible genes was then tested experimentally by Reichard et al. (2012) using Chinese rose bitterling (Rhodeus ocellatus). The study confirmed that females distinguished between males with similar and dissimilar MHC alleles and deposited more eggs with males with a dissimilar MHC profile. However, they also suggested that even in fish the apparent female preference for MHC dissimilarity may be a by-product of other functional loci linked to MHC.

During mate choice, MHC diversity is evaluated by the olfactory system. Females assess males using odor cues carrying information about MHC diversity (i.e., female likes the odor of male with correct combination of MHC alleles). The chemical nature of the MHC-dependent odor in sticklebacks was described by Milinski et al. (2005), who demonstrated experimentally its function in mating choice. The high quality of signals associated with MHC should increase the attractiveness of the male to the choosing female. In sticklebacks, MHC-linked signals are only produced by actively reproducing males, suggesting that the production of these signals is costly to males, but females do not send any MHC signal. However, males also need to validate the MHC signal by another molecule, as was suggested by Milinski et al. (2010).

MHC VARIABILITY IN HYBRID AND POLYPLOID FISH

Very recently, MHC genes were analyzed in a few hybridizing systems and diploid-polyploid complexes of fish. In the case of hybridizing species, in addition to trans-species evolution, MHC diversity also results from genetic introgression, where some MHC alleles of one species introgress into the genome of other species via the hybridization process, as suggested by Wegner & Eizaguirre (2012). They proposed that both parasite-mediated and sexual selections contribute to the introgression of MHC alleles into the genomes of phylogenetically related species, and two mechanisms – negative frequency dependent selection and overdominant selection – can drive the evolution of MHC introgression.

Two phylogenetically related cyprinid species, the native and endemic South-west European nase and the introduced common nase live in two sympatric areas of Southern France (the Durance and Ardeche Rivers, respectively). The presence of their hybrids was documented in both areas (Costedoat et al. 2005, 2007, Šimková et al. 2013a). MHC IIB genes (DAB genes) were analyzed in sympatric and allopatric populations of both species in Southern France in order to investigate the pattern of selection in DAB genes in both species and to elucidate how trans-species evolution and intergeneric hybridization affect the variability of MHC genes. In accordance with previous studies investigating MHC diversity in fish, Šimková et al. (2013a) suggested that the high MHC variability also found in hybridizing cyprinids is a result of several evolutionary mechanisms. In addition, they showed that hybridization plays an important role in increasing MHC variability in the areas where both species live in sympathy and highlighted the potential effect of parasite-mediated selection on MHC genes acting after the introduction of a new parasites species following the introduction of a non-native fish host. However, low genetic diversity among MHC IIB genes hypothetically
related to the potential risk of increased vulnerability to non-native parasites was not observed in the threatened species. In contrast, both species exhibited similar population diversity with respect to MHC IIB genes, whilst at the individual level, invasive species exhibited higher allelic diversity when compared to endemic species. This higher diversity was likely associated with high diversity and abundance of coevolving monogenean parasites, e.g. Dactylogyrus species (Šimková et al. 2012, 2013a). Concerning MHC diversity in hybrids, Eizaguirre et al. (2009) predicted the existence of super-optimal variability of MHC genes in hybrids acting as a mechanism selecting against interspecific hybridization. They proposed that the combination of the MHC alleles of two divergent mates leads to an extreme number of MHC variants in the hybrid genome, and that such extreme variant diversity is responsible not only for the recognition and binding of more foreign antigens, but also for the elimination of self-derived peptides (which are finally eliminated by T-cells). However, the study of endemic South-west European nase and invasive common nase showed that F1 hybrids exhibited an intermediate number of MHC alleles between the parental species (Fig. 1). As ectoparasite intensities (especially monogeneans) were similar between hybrids and endemic species, and both were less infected than invasive species, Šimková et al. (2013a) suggested that the MHC alleles specific to South-west European nase introgressed into the hybrid genomes represent a hybrid advantage, i.e. low hybrid susceptibility to coevolving monogeneans of common nase. Other studies performed on different cyprinid systems, i.e. hybridization between the evolutionarily divergent cyprinids common bream (Abramis brama) and roach (Rutilus rutilus), and hybridization between the phylogenetically closely related species common carp and gibel carp suggest that the individual MHC variability of fish hybrids is more similar to one parental species than to the other, or even lower than the variabilities found in both parental species (unpublished data). In both cases, hybrids were less parasitized, i.e. they exhibited a lower intensity of parasite infection (especially concerning monogeneans) when compared to parental species.

MHC genes were analyzed in diploid-polyploid cyprinid complex to explain the mechanism of coexistence of asexual and sexual fish by Šimková et al. (2013b) using the prediction of the Red-Queen (RQ) hypothesis (Hamilton 1980, Seger & Hamilton 1988, Hamilton et al. 1990). RQ predicts antagonistic coevolution between hosts and their parasites associated with permanent oscillations in their genotype frequencies and resulting in their reciprocal adaptations. Following RQ, the asexual form, due to its low genetic diversity (or the asexual clone representing the most common host genotype) is a target for parasite adaptation. MHC diversity (concerning MHC class I and class IIB) was studied in an asexual-sexual species complex of mollies, including the asexual Amazon molly (Poecilia formosa) and the sexual sailfin molly (Poecilia latipinna) or sexual shortfin molly (Poecilia mexicana) (Schaschl et al. 2008, Lampert et al. 2009). The asexual Amazon molly (like the example of gibel carp described below) reproduces by gynogenesis. This is a form of asexual reproduction which is dependent on the existence of a sexual form. Egg and embryo development is activated by sperm of the sexual form of the same species.
or a phylogenetically closely related sexual species, and the offspring develops parthenogenetically. Lampert et al. (2009) showed high individual allele numbers (more evident for the triploid form of asexual Amazon molly) and reduced genotypic diversity for asexual Amazon molly when compared to sexual shortfin molly. They proposed that the genotypic diversity of MHC is important for pathogen resistance. However, this hypothesis was not later pursued. Schaschl et al. (2008) predicted equal or higher MHC diversity in asexual species when compared to sexual ones if sexual selection is not responsible for MHC diversity. They identified similar MHC I class diversity between asexual and sexual species, but MHC IIB diversity was higher in sexual compared to asexual species. However, they also identified a similar level of positive selection, and previous studies also showed a similar level of parasite infection in both species (Tobler & Schlupp 2005, Tobler et al. 2005). This suggests that high MHC IIB diversity does not confer any advantage to sexual species, i.e. this study did not support an RQ mechanism in an asexual-sexual fish complex.

*Fig. 2.* Parasite species richness (A) and total parasite abundance (B) in gynogens expressing common genotype A, gynogens expressing common genotype B, other gynogens expressing different and rare genotypes and diploid sexuals of gibel carp. Modified from Šimková et al. (2013b).
Later, MHC diversity and potential associations with parasitism were analyzed in a diploid-polyploid complex of gibel carp. Gibel carp – one of the lineages of the *Carassius auratus* complex – is a unique cyprinid model due to the coexistence of gynogenetic and sexual reproduction. Šimková et al. (2013b) analyzed the variability of MHC IIB genes (*DAB*-like alleles) in the gynogenetic form (triploid females) and sexual form (including diploid females and males). They found that the sampled gynogenetic form was composed of specimens expressing two common MHC genotypes and of specimens expressing rare genotypes (*i.e.* the genotypes found in 1 to 3 specimens). In contrast, the sexual form of gibel carp exhibited a wide range of MHC genotypes (and expressed a wide range of *DAB*-like alleles), which is in line with the prediction of sexually-mediated selection increasing MHC diversity. They demonstrated that gynogenetic individuals with two common MHC genotypes (A and B in Fig. 2) were more parasitized than sexual individuals or gynogenetic individuals with the rare genotypes and, at the same time, they revealed different patterns of positively selected sites between the gynogenetic and sexual forms of gibel carp. This finding clearly supports the prediction of the RQ hypothesis, *i.e.* sexual reproduction provides an advantage by creating many different genotypes, this phenomenon conferring resistance to parasites.

**CONCLUSIONS**

High MHC polymorphism in fish is classically explained by two mechanisms – parasite-mediated selection and sexual selection. However, recombination, gene conversion and even neutral evolutionary processes may contribute to MHC diversity in fish. The hypotheses of heterozygote advantage (overdominant selection) or rare allele advantage (negative frequency dependent selection) were empirically supported when studying host-parasite interactions at the level of fish populations, but an intermediate number of *DAB*-like alleles seems to be advantageous for individuals as documented for three-spined stickleback. Behavioral experiments suggest that mating choice determine the MHC diversity in fish offspring as even fish females are able to distinguish between MHC-similar and MHC dissimilar males using olfactory cues. It seems that parasite-mediated and sexual selections contribute to the introgression of MHC alleles into the genomes of phylogenetically related fish species through the hybridization process, and both negative frequency dependent selection and overdominant selection can drive the evolution of MHC introgression. Cyprinid F1 hybrids tend to exhibit an intermediate number of MHC alleles between the parental species, which may represent a hybrid advantage, *i.e.* low hybrid susceptibility to coevolving parasites. Both selections contribute also to the coexistence of asexual-sexual fish complexes.

In this case, the sexual form expressing high variability of MHC variants is favored over asexual form in which the common MHC genotypes seem to be the target of parasite invasion.

**ACKNOWLEDGEMENTS.** – This study was funded by Czech Science Foundation, project no. P505/12/0375 (until 2016) and project no. P505/12/G112 (ECIP) (from 2017). The author would like to thank her colleagues and students – E Ottová, M Košař, M Seiferfová, K Civáňová, S Stierandová, L Gettová and T Pakosta from Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno for participating in the analyses of MHC genes of cyprinid species. The author also would like to thank Matthew Nicholls for his linguistic assistance.

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Vie Milieu, 2017, 67 (2)


Received on August 17, 2017
Accepted on November 10, 2017
Associate editor: Y Dessevises