



Collaboration for
Environmental
Evidence

Collaboration for Environmental Evidence

Systematic Review No. 65

DOES REDUCED MHC DIVERSITY DECREASE VIABILITY OF VERTEBRATE POPULATIONS?

Draft Review Report

Lead Reviewer: *Jacek Radwan*

Postal Address: *Institute of Nature Conservation, Polish Academy of Sciences, Al.
Mickiewicza 33, 31-120 Kraków, Poland*

E-mail Address: *jacek.radwan@uj.edu.pl*

Telephone: *+48 (0)12 6646849*

Fax: *+48 (0)12 6646912*

This is a draft review for consultation. Additional work is in progress. Please do not quote this document or any part therein without prior consent of the authors.

Cover Sheet

Title	Working title: <i>DOES REDUCED MHC DIVERSITY DECREASE VIABILITY OF</i>
Systematic review	N ^o 65
Reviewer(s)	<i>Jacek Radwan, Aleksandra Biedrzycka, Wiesław Babik</i>
Date draft protocol published on website	<i>21st January 2009</i>
Date final protocol published on website	<i>24th March 2009</i>
Date of most recent amendment	
Date of most recent SUBSTANTIVE amendment	
Details of most recent changes	
Contact address	<i>Institute of Nature Conservation, Polish Academy of Sciences, Al. Mickiewicza 33, 31-120 Kraków, Poland, email: jacek.radwan@uj.edu.pl</i>
Sources of support	<i>Polish Academy of Sciences</i>
Conflicts of interest	<i>We are not aware of any conflicts of interests</i>

Summary

1. Background

Pathogens are considered as one of the major extinction factors (Smith et al. 2009; Wilcove et al. 1998). Arguably, depletion of genetic diversity within populations may make them more vulnerable to pathogen assault (Altizer *et al.*, 2003; de Castro and Bolker, 2005; O'Brien and Evermann, 1988). First, inbreeding depression associated with population bottlenecks (Keller and Waller, 2002) may limit the ability of individuals to mount an effective immune response. Indeed, inbreeding has been demonstrated to increase susceptibility to infections (Acevedo-Whitehouse et al., 2003; 2005; Coltman et al., 1999; Imonen et al., 2008; Reid et al., 2007; Ross-Gillespie et al., 2007; Spielman et al., 2004). Second, the loss of variation at genes responsible for resistance to parasites may render populations more susceptible to infection. This argument applies to highly polymorphic vertebrate Major Histocompatibility Complex (MHC) genes, coding for proteins presenting pathogen-derived antigens to T-cells, thus initiating the adaptive immune response (Janeway et al., 2004). Hughes (1991) suggested that retention of variation in these genes is an essential element of effective conservation programmes, but this argument remains controversial (Hedrick, 2001). Apart from MHC, other polymorphic genes can influence the effectiveness of defences against pathogens (Acevedo-Whitehouse and Cunningham, 2006). Here however we concentrate on MHC genes only, as they are the most polymorphic genes known in vertebrates, and their function and evolution is better understood than that of other genes involved in the immune response.

2. Objectives

To answer the major question whether reduced MHC diversity decreases viability of vertebrate populations? This question has three components which can be formulated into more specific questions: (1) can drift render MHC loci effectively neutral and thus reduce their diversity in populations? (2) can reduced variation at MHC increase population-level parasite load or prevalence of disease? (3) Does reduced variation at MHC increase probability of population extinction?

3. Methods

Multiple electronic sources were searched using several Boolean expressions. The relevance of a study was first assessed by reading the title and abstract, and then by reading full texts of the papers considered relevant. Two reviewers must reach consensus regarding which data are relevant. Searches were performed only in English. Due to the small number of identified studies, qualitative analysis was performed and results tabulated.

4. Main results

Majority of studies comparing levels of MHC diversity across populations with that of neutral variation have found a significant correlation. This indicates that on the short time scale MHC variation is shaped predominately by demographic processes rather than by selection. Nevertheless, some tests of selection suggest the role of selection in shaping MHC allele frequencies. In two of twelve species included in Table 1 evidence of balancing selection and not of drift shaping MHC variation was found. In five species the

evidence was mixed, indicating that both drift and selection may have an impact, and five studies found evidence of drift only. In one of the two positive studies the evidence of positive selection was found only in a fraction of populations.

We have found only a single study relating infection to MHC diversity across populations; no evidence that MHC variation was associated with mortality caused by the bacterial pathogen was found in this study.

We have not found any studies reporting populations or species that have gone extinct following reduction of MHC variations.

5. Conclusions

Implications for conservation Given the uncertainty about the role of MHC variation for population viability, avoidance of inbreeding as the main aim of genetic restoration programmes seems reasonable, especially since inbreeding depression has well documented detrimental effects on fitness, including impairment of the immune response. Thus, it seems rational to recommend inbreeding avoidance as a priority in cases where it would conflict with retention of maximum MHC variation. Natural populations harbouring the most MHC variation will also usually have more genome-wide diversity, therefore the protection of both types of genetic diversity can be achieved simultaneously.

Implications for research Despite balancing selection shaping MHC polymorphism in the long term, MHC variation is often substantially reduced due to genetic drift acting in bottlenecked and fragmented populations. However, whether such a loss poses a threat to the survival of populations remains unclear. The scarcity of direct evidence for the impact of MHC diversity on the survival prospects of populations, coupled with examples of long-term survival of populations despite reduced MHC polymorphism may suggest that MHC diversity is not as serious a concern in conservation as some authors have suggested. However, as the causes of past extinction events are usually uncertain, the evidence is likely to be unbalanced: it is easier to document survival, than extinction of species with depleted MHC diversity. There is thus an imperative need for data that could indirectly reveal the possible consequences of MHC diversity for population viability. In particular, we need more data on the impact of MHC allelic richness on the abundance of parasites and prevalence of disease in populations. Such data, although indirect, are much easier to obtain than the data relating MHC variation to actual extinction events. Efforts should be made to control for genome-wide inbreeding. Complementary research should assess the role of pathogens in shaping population dynamics.

Main Text

1. Background

Pathogens are considered as one of the major extinction factors (Smith et al. 2009; Wilcove et al. 1998). Arguably, depletion of genetic diversity within populations may make them more vulnerable to pathogen assault (Altizer et al. 2003; de Castro and Bolker 2005; O'Brien and Evermann 1988). First, inbreeding depression associated with population bottlenecks (Keller and Waller 2002) may limit the ability of individuals to mount an effective immune response. Indeed, inbreeding has been demonstrated to increase susceptibility to infections (Acevedo-Whitehouse et al. 2003; 2005; Coltman et al. 1999; Ilmonen et al. 2008; Reid et al. 2007; Ross-Gillespie et al. 2007; Spielman et al. 2004). Second, the loss of variation at genes responsible for resistance to parasites may render populations more susceptible to infection. This argument applies to highly polymorphic vertebrate Major Histocompatibility Complex (MHC) genes, coding for proteins presenting pathogen-derived antigens to T-cells, thus initiating the adaptive immune response (Janeway et al. 2004). Hughes (1991) suggested that retention of variation in these genes is an essential element of effective conservation programmes, but this argument remains controversial (Hedrick 2001). Apart from MHC, other polymorphic genes can influence the effectiveness of defences against pathogens (Acevedo-Whitehouse and Cunningham 2006). Here however we concentrate on MHC genes only, as they are the most polymorphic genes known in vertebrates, and their function and evolution is better understood than that of other genes involved in the immune response. Despite this, as shown in our review, there is few data linking MHC diversity to population viability and some of it is equivocal. We hope that by drawing attention to gaps in our knowledge, our review will stimulate research that will facilitate better design of conservation programmes.

Adaptive significance of MHC variation.

MHC codes for the most polymorphic genes in vertebrates (Garrigan and Hedrick 2003), some of which (A, B within MHCI and DRB within MHC II) have hundreds of alleles described in human populations (<http://www.ebi.ac.uk/imgt/hla/stats.html>), and dozens of alleles typically found in large vertebrate populations. Most variation in individual MHC genes is concentrated in regions coding for extracellular domains forming a groove that binds antigens from either intracellular (MHC class I) or extracellular (MHC II) pathogens (Hedrick and Kim 1999). Polymorphism within these peptide-binding regions is thought to be maintained by some form of balancing selection imposed by pathogens. Fast evolving parasites may adapt to the most common host genotype and escape presentation of their antigens to the adaptive immune system of the host. Rare allelic variants of MHC genes to which parasites are unlikely to adapt would thus be favoured by negatively frequency-dependent selection (Borghans et al. 2004; Snell 1968). Additionally, heterozygote advantage in resistance to parasites can contribute to polymorphism at MHC loci, as heterozygotes should be able to present a broader range of antigens (Doherty and Zinkernagel 1975; Hughes and Nei 1992; Takahata and Nei 1990). Both these mechanisms would result in retention of allelic lineages over long evolutionary timescales, resulting in transspecific polymorphism (Klein 1987), and in the establishment of novel alleles that arise via mutation or micro-recombination. Indeed, in most species

investigated so far, positions in MHC sequences coding for residues involved in binding antigens consistently show an excess of nonsynonymous over synonymous substitutions, which is the hallmark of positive selection for amino acid substitutions (Garrigan and Hedrick 2003; Piertney and Oliver 2006; Sommer 2005). There is growing evidence for the association of MHC genotypes or individual alleles and susceptibility to infection (Bonneaud et al. 2006a; Deter et al. 2008; Schwensow et al. 2007; reviewed in Sommer 2005) confirming that pressure from parasites is the primary source of selection on MHC. Indeed, in sticklebacks, parasite diversity was positively associated with MHC diversity across populations (Wegner et al. 2007). As no correlation of parasite load with microsatellite diversity was detected, it seems likely that it is not the MHC diversity that affected population-level parasite load, but conversely, it is the prevalence of parasites that has promoted maintenance of high MHC variation. Similarly, Dionne et al. (2007) observed that MHC diversity changed with latitudinal temperature gradient in Atlantic salmon, but also with bacterial diversity in the environment. Their analysis suggested that bacterial diversity is predominant in shaping MHC variability.

Balancing selection can also result from MHC-dependent choice of mates (Apanius et al. 1997). Several species show preferences for MHC-dissimilar mates (Olsson et al. 2003; Radwan et al. 2008; Schwensow et al. 2008; Yamazaki et al. 1976), which may be an adaptation to avoid mating with relatives or a way of producing MHC-heterozygous progeny, immune to a wider range of pathogens (Milinski 2006; Penn 2002). However, as preference for MHC-dissimilar mates does not seem to be the rule (e.g. Bonneaud et al. 2006b; Lampert et al. 2006; Miller et al. 2001; Paterson et al. 1998), it seems likely that pressure from parasites is the primary source of selection on MHC.

MHC in conservation

O'Brien and Everman (1988) suggested that the high susceptibility of cheetah and other bottlenecked species to diseases may result from the loss of variation at genes responsible for defence against diseases, such as MHC genes. As discussed above, there is growing evidence for an association of MHC types with susceptibility to infection, which seems to support the hypothesis of O'Brien and Everman. In large populations, genotypes resistant to many pathogens should be present due to ample MHC variation. However, in bottlenecked populations rare MHC alleles may be lost due to strong random drift, thus compromising the ability of a population to respond to fast-evolving parasites: none of the few alleles remaining may be able to present antigens of a novel parasite type. Furthermore, parasites are expected to adapt to the most common host genotypes. For example, the human HLA-A11 allele confers resistance to Epstein-Barr disease, but only in populations in which it is rare, implying that an increase in frequency of the resistant allele facilitates adaptation in the parasites (de Campos-Lima et al. 1993). Consequently, parasites may evolve ways to evade host immune systems faster in populations with limited MHC diversity. Finally, the recovery of MHC variation, which to some extent arises via micro-recombination between existing alleles within MHC loci (Richman et al. 2003), can be hindered in bottlenecked populations due to their lower effective recombination rate. However persuasive the arguments listed above, evidence for the effect of MHC diversity on population viability is needed before reliable recommendations for conservation programmes are made. After reviewing such evidence over a decade ago, Edwards and Potts (1996) concluded that “None of the studies [on species exhibiting low MHC polymorphism] identified the causes of low variability

experimentally, and none has established causal relationship between low MHC variability and disease susceptibility”. Here, we review studies that have accumulated since the article by Edwards and Potts (1996) was published. We concentrated on three questions (1) whether drift can render MHC loci effectively neutral and thus reduce their diversity in populations; (2) whether reduced variation at MHC increases population-level parasite load or prevalence of disease; (3) whether reduced variation at MHC increases probability of population extinction.

2. Objectives

2.1 Primary objective

Primary objective of the review is to answer the question “Does reduced MHC diversity decrease viability of vertebrate populations?”

2.2 Secondary objective (*if applicable*)

Answering the following, more specific questions should help to address the primary objective: (1) can drift render MHC loci effectively neutral and thus reduce their diversity in populations? (2) can reduced variation at MHC increase population-level parasite load or prevalence of disease? (3) does reduced variation at MHC increase probability of population extinction?

3. Methods

3.1 Search strategy

Databases:

- ISI Web of Knowledge (1996-2009)
- Scopus (since the beginning)

We did not consider additional databases or attempt to search ‘grey literature’ because to be included in our review studies had to fulfil strict quality criteria, which are likely to be met in mainly by papers published in impacted journals.

Search terms:

To address the specific questions:

- (1) “MHC AND drift”
- (2) “MHC AND (diversity OR variation) AND (infection OR disease OR parasite* OR pathogen*)”
- (3) ”MHC AND (extinction OR population survival OR population viability)”

3.3 Study inclusion criteria

- **Relevant subject(s):** Populations of any vertebrate species
- **Types of intervention:** Decreased MHC diversity (e.g. through bottleneck)
- **Types of comparator:** No decrease in MHC diversity
- **Types of outcome:** Risk of extinction
- **Types of study:** The extracted data should include tests of neutrality of MHC genes in populations which have undergone a decrease in population size, ; parasite/pathogen loads or disease prevalence in populations differing in the

level of MHC variation; recorded extinctions of species with different levels of mhc variation;

- **Potential reasons for heterogeneity:** variation in methodologies used to test for selection

3.4 Study quality assessment, data extraction and data synthesis

The following criteria were used to assess the quality of studies:

- Number of populations
- Tests of neutrality of MHC variation
- Tests for signatures of historical balancing selection
- Availability MHC sequence data
- Assessment of decrease MHC variation in bottlenecked populations?

The relevance of a study was first assessed by reading the title and abstract, and then by reading full texts of the papers considered relevant. Two reviewers must reach consensus regarding which data are relevant. Searches were performed only in English. Because the number of relevant studies identified during our searches was definitively too small for formal metaanalysis, we present qualitative synthesis in the form of tabulation (Table 1).

4. Results

4.1 Review statistics

Searches of the databases gave the following results :

Question 1 ISI: 63, Scopus: 86, 13 (ca. 15%) studies were found relevant

Question 2 ISI: 688, Scopus: 1166 only ONE (less than 0.1%) was found relevant

Question 3 ISI: 28, Scopus 38, NONE was found relevant

4.2 Qualitative synthesis

Question (1) Thirteen studies were identified which tested whether drift can render MHC variation, normally under selection, neutral, due to reduction of population size (bottleneck).

In 10 of 13 studies evidence that drift has been shaping MHC variation was found. This was manifested as a correlation between variation at MHC and variation at neutral loci. In five species the evidence was mixed (different tests yielded disparate conclusions), indicating that both drift and selection may have an impact, and five studies found evidence of drift only. In one of the two positive studies, the sockeye salmon, the evidence of positive selection was found only in a fraction of populations (Table 1). Overall, MHC alleles seem to often be affected by drift even if reduction of population size is not extreme.

Question (2) We have found only a single study relating infection to MHC diversity across populations. Giese and Hedrick (2003) found no evidence that MHC variation was associated with mortality caused by the bacterial pathogen *Listonella anguillarum* in the endangered gila topminnow. The same was true of other measures of genetic diversity, including variation at microsatellite loci.

Question (3) We did not find any study addressing the question and meeting quality criteria.

4.6 Outcome of the review

The qualitative analysis of studies examining the question whether drift can render MHC loci, normally under selection, neutral in bottlenecked populations the answer is generally positive. We detected the evidence of the influence of drift in most of 13 analyses cases. However the number of studies fulfilling criteria of the review is low and the issue deserves further research.

As to the second question whether decreased MHC variation increases susceptibility to infection we were able to identify only one study, which did not detect the effect.

We did not find any study directly assessing the third question, whether low MHC variation can lead to extinction. However, we want to stress that addressing this question is difficult and biased. While it is possible to determine MHC variation in species that have survived despite bottlenecks, there is usually no way of telling if species which went extinct in the past did so because of low MHC variation.

Altogether our review identified serious knowledge gaps and thus should stimulate further research.

5. Discussion

5.1 MHC diversity and genetic drift

Alleles will be effectively neutral if $s < 1/2N_e$, where s is the selection coefficient and N_e is the effective population size. Thus, MHC alleles in even moderate frequencies will be subject to loss due to drift if population size is small or if balancing selection acting on MHC is relatively weak. A number of species that have experienced extreme population bottlenecks do indeed show depletion of variation at MHC, e.g. cheetah (O'Brien et al. 1985), Eurasian beaver (Babik et al. 2005; Ellegren et al. 1993), European bison (Radwan et al. 2007), giant panda (Zhu et al. 2007), Seychelles warbler (Hansson and Richardson 2005), mountain goat (Mainguy et al. 2007) and Galapagos penguin (Bollmer et al. 2007). In most of these cases, the DNA sequences of the MHC genes show signatures of historical positive selection, so limited MHC variation can be attributed to drift, rather than to the lack of selection on MHC (Babik et al. 2005; Mikko and Andersson 1995; Radwan et al. 2007).

However, strong balancing selection is thought to be able to resist drift even in drastically bottlenecked populations (Aguilar et al. 2004; van Oosterhout et al. 2006).

The study of moderately bottlenecked fragmented populations within species offers more stringent tests of the role of drift in shaping MHC variation. Several recent studies compared levels of MHC diversity across populations with that of neutral variation, and most have found a significant correlation (Table 1). This indicates that on the short time scale MHC variation is shaped predominately by demographic processes rather than by selection. Nevertheless, some tests of selection, such as these of Ewens-Watterson or Tajima, suggest the role of selection in shaping MHC allele frequencies (Table 1).

Rather than selection, population demographic history may account for the significant outcomes of neutrality tests (Nielsen 2005), although it can be to some extent corrected for by running simultaneous test on neutral markers (e.g. Bryja et al. 2007). A more rigorous test of the role of selection is offered by the F_{ST} outlier analysis (Beaumont

and Nichols 1996). The rationale behind this analysis is that demographic processes affect neutral loci, distributed over the genome, in the same way, leading to a single expectation for the level of population differentiation (measured by F_{ST}). Loci which fall outside of confidence intervals of the neutral F_{ST} values are likely to be under selection – diversifying if they show significantly higher F_{ST} and spatially uniform if their F_{ST} is lower than neutral expectations (Beaumont 2005). The spatially uniform selection is a likely result of balancing selection, preventing the loss of rare alleles and homogenising their frequencies across populations, whereas diversifying selection may result from directional selection, which could enhance the loss of genetic diversity rather than prevent it. Applications of this test to microsatellite and MHC variation revealed significant deviation from neutrality in two of six studies (Table 1). In both these studies, however, F_{ST} outlier analysis indicated action of diversifying selection. Overall, MHC alleles seem to often be affected by drift even if reduction of population size is not extreme.

MHC diversity and susceptibility to infection.

We have found only a single study relating infection to MHC diversity across populations. Giese and Hedrick (2003) found no evidence that MHC variation was associated with mortality caused by the bacterial pathogen *Listonella anguillarum* in the endangered gila topminnow. The same was true of other measures of genetic diversity, including variation at microsatellite loci. As results of a single study are hard to generalise, we summarise below other studies that provide some suggestive data, and we highlight the need for further research.

Perhaps the most convincing is the case of the Tasmanian devil, reported recently by Siddle et al. (2007), in which the spread of a transmissible tumour is apparently facilitated by very low variation at MHC I. The tumour, transferred between individuals by biting, may be considered as an allograft, and should thus be rejected by the host immune system following recognition of foreign MHC. The lack of rejection might result from the down-regulation of classical MHC molecule expression by the tumour, but Siddle et al. found no evidence of this; rather, because of low MHC I variation, the tumour is often perceived as “self” by the host immune system.

While MHC monomorphism prevents graft rejection, it may still allow presentation of a large number of antigens from pathogens due to the promiscuity of peptide binding by MHC molecules (Nikolich-Zugich et al. 2004). Thus, the susceptibility of populations with limited MHC variation to an unusual transmissible tumour may not be representative of threats posed by pathogens to population survival. Mainguy et al. (2007) found no evidence for increased susceptibility to disease in a bottlenecked population of mountain goats, in which only two MHC DRB alleles were retained. On the other hand, high mortality in a breeding colony of cheetah caused by coronavirus-associated feline infectious peritonitis has been attributed to reduced genetic variation at MHC, ascertained in this species (O'Brien et al. 1985). However, general inbreeding may have also brought about the same outcome (Fig. 1). Indeed, separation of the effect of general inbreeding from that of depletion of MHC variation may often be difficult. In several studies infections were shown to be correlated with decreased genetic diversity, although variation at MHC had not been directly assessed. The stability of populations of critically endangered Ethiopian wolf (*Canis simensis*) is affected by rabies virus epidemics, particularly likely to eradicate smaller populations (van de Bildt et al. 2002). As small populations are expected to show lower levels of genetic variation, this suggests a link

between susceptibility to infection and genetic variation. Pearman and Garner (2005) compared susceptibility to infection with FV3 (frog virus 3) among populations of the Italian agile frog *Rana latastei*. They found that populations with lower microsatellite diversity suffered higher mortality imposed by this emerging pathogen. On a smaller scale, a bottlenecked population of a New Zealand robin *Petroica australis* had similar parasite load, but lower immune response compared to a more diverse source population (Miller and Lambert 2004). Whiteman et al. (2006) found a significant association of minisatellite diversity and louse abundance across populations of the Galapagos hawk (*Buteo galapagoensis*). They also found that genetic diversity correlated with the level of natural antibodies (Nabs), which in turn was also correlated with louse abundance. As expression of Nabs is constitutive and not antigen-dependent, the increased susceptibility of genetically depauperate populations to louse infestation is likely due to factors other than depletion of MHC variation. Whether this is also true for other cases of inverse correlations between genetic diversity (including MHC diversity) and infection susceptibility remains to be investigated. Future studies should make an effort to separate effects of genome-wide inbreeding from the effect of reduced MHC variation. In natural populations this will often be difficult, as MHC and neutral variation are often correlated (Table 1), unless strong selection will maintain substantial MHC diversity despite low N_e (see previous section). Experimental approaches (e.g. Giese and Hedrick 2003) are potentially very informative, but limited to species that can be subjected to experimental breeding.

Does MHC variation affect population survival?

We found no studies reporting populations or species that have gone extinct following reduction of MHC variations. There is a number of studies reporting a limited MHC variation in species that have undergone population bottlenecks, but yet the populations survived and even increased in number (reviewed below). However, we want to stress that this data set is likely to be heavily biased. While it is possible to determine MHC variation in species that have survived despite bottlenecks, there is usually no way of telling if species which went extinct in the past did so because of low MHC variation. Thus, the examples of species whose MHC variation has been depleted during extreme bottlenecks, but which nevertheless were able to recover and increase in numbers, can be taken as evidence that not all such species are bound to go extinct, but not as a proof that MHC diversity does not affect probability of extinction.

Several studies concluded that MHC variation has little effect on population dynamics on the short timescale (Babik et al. 2005; Ellegren et al. 1993; Mainguy et al. 2007; Mikko and Andersson 1995; Weber et al. 2004). An extreme example is provided by the Scandinavian population of Eurasian beaver, lacking variation at MHC altogether (Ellegren et al. 1993). Despite this, the population size increased from about 100 to more than 150 000 during the 20th century (Halley and Rosell 2002). The speculation that beavers may have experienced little selection on MHC (Ellegren et al. 1993) has been rejected by data from other relict Eurasian beaver populations. Babik et al. (2005) have found that while six out of seven of these populations were monomorphic, each was fixed for a different MHC II DRB allele. This pattern of variation, and a strong signal of historical selection acting on DRB sequences, indicate that MHC variation was lost due to the extreme bottleneck the species experienced at the end of the 19th century (Babik et al. 2005). Most of these populations recovered after the species was granted legal protection

(Halley and Rosell 2002; Nolet and Rosell 1998), suggesting that populations can thrive despite MHC monomorphism. However, a century that has passed since the bottleneck may be too brief a period for the conclusion that MHC variation does not affect population dynamics. A longer timescale is provided by species whose genetic variation was reduced during glaciations. In great crested newts, ample variation at MHC II has been retained in the southern glacial refugium, but northern populations, founded during postglacial expansion, harbour only two expressed alleles. Despite such limited variation, populations in the expansion area have been viable for several thousands of years (Babik *et al.* 2009). In moose MHC variation was probably lost during a bottleneck that occurred about 100 000 years ago and since then new mutations accumulated and additional alleles have apparently arisen via microrecombination (Mikko and Andersson 1995). Most of the variation in the newly emerged MHC alleles is non-synonymous, indicating that they were quickly driven to high frequencies via positive Darwinian selection. This is an informative case indicating that loss of MHC is not necessarily conducive to extinction, but this does not imply a lack of selection on MHC. This is to be expected if selection resulting from parasites arises from reduced competitiveness of infected individuals instead of from mortality (Babik *et al.* 2005). Alternatively, mortality may be of compensatory, rather than of additive character (or soft, rather than hard), i.e. parasites may cause the death of the proportion of infected individuals that exceeds the carrying capacity of their environment (Combes 1996, 1997). This scenario would result in long-term selection, evident in sequences, but with little effect on population dynamics. Indeed, recent reviews concluded that despite infectious disease being considered one of five major extinction factors (Wilcove *et al.* 1998), there is little hard evidence to support this conviction (de Castro and Bolker 2005; Smith *et al.* 2006). Extinction was attributed, at least partially, to infectious disease in only 3.5% of species reported extinct in the last 500 years by IUCN Red list, but in no case was it listed as the single cause (Smith *et al.* 2006). The authors emphasize, however, that there is a large degree of uncertainty concerning the inferred causes of extinction reported by the IUCN, so the role of parasites in extinction should not be prematurely dismissed. Moreover, some convincingly documented examples of local extinction or near-extinction caused by disease have been documented (reviewed in de Castro and Bolker 2005; McCallum and Dobson 2002), and there is growing evidence that infections by a chytrid fungus is one of the major causes of dramatic amphibian declines observed in the last three decades (Lips *et al.* 2008). Even if selection induced by parasites is hard, population survival might not be compromised by the reduction of MHC variation if the variants retained show much of the original functional variation. Indeed, Hedrick *et al.* (2002) argued that species threatened with extinction tend to retain highly divergent allelic variants. In the red wolf, only 4 allelic variants are found, but their divergence is high. Simulations based on 27 sequences found in red wolves and in related Mexican wolves and coyotes have shown that this level of divergence was unlikely to have arisen by chance. If this is a general pattern, then the potential of endangered species to respond to pathogen assault may be higher than the small number of retained alleles suggest, i.e. due to the promiscuity of peptide binding, a few divergent alleles may be able to bind antigens from a wide range of parasites.

Review limitations

We deliberately restricted our searches to two large databases: ISI Web of Knowledge and Scopus. These databases report mainly high quality papers from peer-reviewed journals. We cannot exclude that a small number of relevant studies was missed by restricting our search to these high quality sources. However, screening reference list in indexed publications should alleviate this problem. Concerning the publication bias it is difficult to assess, however it does not seem probable that studies meeting our strict criteria should be underrepresented in publications depending on their results. An inherent limitation of our review is the biased nature of the data needed to answer question (3) does reduced variation at MHC increase probability of population extinction? While it is possible to determine MHC variation in species that have survived despite bottlenecks, and such species are indeed known, there is usually no way of telling if species which went extinct in the past did so because of low MHC variation. Possibly, as long term data on the extinction of populations will accumulate it will be possible to present correlation or perhaps even infer causal relationship between the extinction rate and depletion of MHC variation.

6. Reviewers' Conclusions

6.1 Implications for conservation

Hughes (1991) argued that captive breeding programmes should be designed to protect diversity at MHC. His argument may be summarised as follows: although for a given population size the loss of average heterozygosity may not be very high, variation at some individual loci may be lost. Loss of variation at many loci would not matter, as allelic variants are most often neutral. Therefore, we should concentrate on loci whose adaptive significance is well understood, such as MHC loci. Hedrick (2001), however, pointed out that if captive breeding programs aim to preserve variation at a single locus, families with rare alleles will be over-represented and this may lead to increased genome-wide homozygosity, exposing deleterious mutations and causing inbreeding depression. Thus, he recommended avoidance of inbreeding as the main aim of genetic restoration programmes. Given the uncertainty about the role of MHC variation for population viability, this recommendation seems reasonable, especially since inbreeding depression has well documented detrimental effects on fitness, including impairment of the immune response (reviewed above). Thus, it seems rational to recommend inbreeding avoidance as a priority in cases where it would conflict with retention of maximum MHC variation. However, breeding programs designed to avoid inbreeding will tend to retain much of the MHC variation, so that such conflicts will rarely be severe. Furthermore, natural populations harbouring the most MHC variation will also usually have more genome-wide diversity (Table 1), therefore the protection of both types of genetic diversity can be achieved simultaneously.

6.2 Implications for research

There is ample evidence for long-term balancing selection acting on peptide-binding regions of MHC, and associations between MHC types and susceptibility to infection have been documented in numerous cases. Despite balancing selection shaping MHC polymorphism in the long term, MHC variation is often substantially reduced due to genetic drift acting in bottlenecked and fragmented populations. However, whether such a loss poses a threat to the survival of populations remains unclear. The scarcity of direct evidence for the impact of MHC

diversity on the survival prospects of populations, coupled with examples of long-term survival of populations despite reduced MHC polymorphism may suggest that MHC diversity is not as serious a concern in conservation as some authors have suggested. However, as the causes of past extinction events are usually uncertain, the evidence is likely to be unbalanced: it is easier to document survival, than extinction of species with depleted MHC diversity. Furthermore, most convincing examples of long-term survival of species come from northern populations (Babik et al. 2005; Ellegren et al. 1993; Mainguy et al. 2007; Mikko and Andersson 1995). This may be a consequence parasite pressure decreasing with latitude (Guernier et al. 2004; Mainguy et al. 2007), and so these examples may not be representative of the overall impact of MHC variation on population survival. There is thus an imperative need for data that could indirectly reveal the possible consequences of MHC diversity for population viability. In particular, we need more data on the impact of MHC allelic richness on the abundance of parasites and prevalence of disease in populations. Such data, although indirect, are much easier to obtain than the data relating MHC variation to actual extinction events. Efforts should be made to control for genome-wide inbreeding. Complementary research should assess the role of pathogens in shaping population dynamics (see de Castro and Bolker 2005; Smith et al. 2006).

7. Acknowledgements

8. Potential Conflicts of Interest and Sources of Support

We are not aware of any conflicts of interest.

9. References

- Acevedo-Whitehouse, K., Cunningham, A.A., 2006. Is MHC enough for understanding wildlife immunogenetics? *Trends Ecol Evol* 21, 433-438.
- Acevedo-Whitehouse, K., Gulland, F., Greig, D., Amos, W., 2003. Disease susceptibility in California sea lions. *Nature* 422, 35-35.
- Acevedo-Whitehouse, K., Vicente, J., Gortazar, C., Hofle, U., Fernandez-De-Mera, I.G., Amos, W., 2005. Genetic resistance to bovine tuberculosis in the Iberian wild boar. *Molecular Ecology* 14, 3209-3217.
- Aguilar, A., Garza, J.C., 2006. A comparison of variability and population structure for major histocompatibility complex and microsatellite loci in California coastal steelhead (*Oncorhynchus mykiss* Walbaum). *Molecular Ecology* 15, 923-937.
- Aguilar, A., Roemer, G., Debenham, S., Binns, M., Garcelon, D., Wayne, R.K., 2004. High MHC diversity maintained by balancing selection in an otherwise genetically monomorphic mammal. *Proceedings of the National Academy of Sciences of the United States of America* 101, 3490-3494.
- Alcaide, M., Edwards, S.V., Negro, J.J., Serrano, D., Tella, J.L., 2008. Extensive polymorphism and geographical variation at a positively selected MHC class IIB gene of the lesser kestrel (*Falco naumanni*). *Molecular Ecology* 17, 2652-2665.
- Altizer, S., Harvell, D., Friedle, E., 2003. Rapid evolutionary dynamics and disease threats to biodiversity. *Trends in Ecology & Evolution* 18, 589-596.

- Apanius, V., Penn, D., Slev, P.R., Ruff, L.R., Potts, W.K., 1997. The nature of selection on the major histocompatibility complex. *Critical Reviews in Immunology* 17, 179-224.
- Babik, W., Durka, W., Radwan, J., 2005. Sequence diversity of the MHC DRB gene in the Eurasian beaver (*Castor fiber*). *Molecular Ecology* 14, 4249-4257.
- Babik, W., Pabijan, M., Arntzen, J.W., Cogalniceanu, D., Durka, W., Radwan, J., 2009. Long-term survival of a urodele amphibian despite depleted major histocompatibility complex variation. *Molecular Ecology* 18, 769-781.
- Babik, W., Pabijan, M., Radwan, J., 2008. Contrasting patterns of variation in MHC loci in the Alpine newt. *Molecular Ecology* 17, 2339-2355.
- Beaumont, M.A., 2005. Adaptation and speciation: what can F_{st} tell us? *Trends in Ecology & Evolution* 20, 435-440.
- Beaumont, M.A., Nichols, R.A., 1996. Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London Series B-Biological Sciences* 263, 1619-1626.
- Bollmer, J.L., Vargas, F.H., Parker, P.G., 2007. Low MHC variation in the endangered Galapagos penguin (*Spheniscus mendiculus*). *Immunogenetics* 59, 593-602.
- Bonneaud, C., Chastel, O., Federici, P., Westerdaal, H., Sorci, G., 2006a. Complex MHC-based mate choice in a wild passerine. *Proceedings of the Royal Society B-Biological Sciences* 273, 1111-1116.
- Bonneaud, C., Perez-Tris, J., Federici, P., Chastel, O., Sorci, G., 2006b. Major histocompatibility alleles associated with local resistance to malaria in a passerine. *Evolution* 60, 383-389.
- Borghans, J.A.M., Beltman, J.B., De Boer, R.J., 2004. MHC polymorphism under host-pathogen coevolution. *Immunogenetics* 55, 732-739.
- Bryja, J., Charbonnel, N., Berthier, K., Galan, M., Cosson, J.F., 2007. Density-related changes in selection pattern for major histocompatibility complex genes in fluctuating populations of voles. *Molecular Ecology* 16, 5084-5097.
- Campos, J.L., Posada, D., Moran, P., 2006. Genetic variation at MHC, mitochondrial and microsatellite loci in isolated populations of Brown trout (*Salmo trutta*). *Conservation Genetics* 7, 515-530.
- Coltman, D.W., Pilkington, J.G., Smith, J.A., Pemberton, J.M., 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* 53, 1259-1267.
- Combes, C., 1996. Parasites, biodiversity and ecosystem stability. *Biodiversity and Conservation* 5, 953-962.
- Combes, C., 1997. Fitness of parasites: Pathology and selection. *International Journal for Parasitology* 27, 1-10.
- de Campos-Lima, P.O., Gavioli, R., Zhang, Q.J., Wallace, L.E., Dolcetti, R., Rowe, M., Rickinson, A.B., Masucci, M.G., 1993. HLA-A11 epitope loss isolates of Epstein-Barr virus from a highly A11+ population. *Science* 260, 98-100.
- de Castro, F., Bolker, B., 2005. Mechanisms of disease-induced extinction. *Ecology Letters* 8, 117-126.
- Deter, J., Bryja, J., Chaval, Y., Galan, M., Henttonen, H., Laakkonen, J., Voutilainen, L., Vapalahti, O., Vaheri, A., Salvador, A.R., Morand, S., Cosson, J.F., Charbonnel, N., 2008. Association between the DQA MHC class II gene and Puumala virus infection in *Myodes glareolus*, the bank vole. *Infection Genetics and Evolution* 8, 450-458.
- Dionne, M., Miller, K.M., Dodson, J.J., Caron, F., Bernatchez, L., 2007. Clinal variation in MHC diversity with temperature: evidence for the role of host-pathogen interaction on local adaptation in Atlantic salmon. *Evolution* 61, 2154-2164.

- Doherty, P.C., Zinkernagel, R.M., 1975. Enhanced immunological surveillance in mice heterozygous at H-2 gene complex. *Nature* 256, 50-52.
- Ekblom, R., Saether, S.A., Jacobsson, P., Fiske, P., Sahlman, T., Grahn, M., Kalas, J.A., Hoglund, J., 2007. Spatial pattern of MHC class II variation in the great snipe (*Gallinago media*). *Molecular Ecology* 16, 1439-1451.
- Ellegren, H., Hartman, G., Johansson, M., Andersson, L., 1993. Major histocompatibility complex monomorphism and low-levels of DNA-fingerprinting variability in a reintroduced and rapidly expanding population of beavers. *Proceedings of the National Academy of Sciences of the United States of America* 90, 8150-8153.
- Garrigan, D., Hedrick, P.W., 2003. Perspective: Detecting adaptive molecular polymorphism: Lessons from the MHC. *Evolution* 57, 1707-1722.
- Giese, A.R., Hedrick, P.W., 2003. Genetic variation and resistance to a bacterial infection in the endangered Gila topminnow. *Animal Conservation* 6, 369-377.
- Guernier, V., Hochberg, M.E., Guegan, J.F.O., 2004. Ecology drives the worldwide distribution of human diseases. *Plos Biology* 2, 740-746.
- Halley, D.J., Rosell, F., 2002. The beaver's reconquest of Eurasia: status, population development and management of a conservation success. *Mammal Review* 32, 153-178.
- Hansen, M.M., Skaala, O., Jensen, L.F., Bekkevold, D., Mensberg, K.L.D., 2007. Gene flow, effective population size and selection at major histocompatibility complex genes: brown trout in the Hardanger Fjord, Norway. *Molecular Ecology* 16, 1413-1425.
- Hansson, B., Richardson, D.S., 2005. Genetic variation in two endangered *Acrocephalus* species compared to a widespread congener: estimates based on functional and random loci. *Animal Conservation* 8, 83-90.
- Hedrick, P.W., 2001. Conservation genetics: where are we now? *Trends in Ecology & Evolution* 16, 629-636.
- Hedrick, P.W., Kim, T.J., 1999. Genetics of complex polymorphisms: parasites and maintenance of MHC variation, In *Evolutionary Genetics from Molecules to Morphology*. eds R.S. Singh, C.K. Krimbas, pp. 204-234. Cambridge University Press, New York.
- Hedrick, P.W., Lee, R.N., Garrigan, D., 2002. Major histocompatibility complex variation in red wolves: evidence for common ancestry with coyotes and balancing selection. *Molecular Ecology* 11, 1905-1913.
- Hughes, A.L., 1991. MHC polymorphism and the design of captive breeding programs. *Conservation Biology* 5, 249-251.
- Hughes, A.L., Nei, M., 1992. Maintenance of MHC Polymorphism. *Nature* 355, 402-403.
- Ilmonen, P., Penn, D.J., Damjanovich, K., Clarke, J., Lamborn, D., Morrison, L., Ghotbi, L., Potts, W.K., 2008. Experimental infection magnifies inbreeding depression in house mice. *Journal of Evolutionary Biology* 21, 834-841.
- Janeway, C.A., Travers, P., Walport, D., Shlomchik, M.J., 2004. *Immunobiology: The Immune System in Health and Disease*. Garland Publishing, New York.
- Keller, L.F., Waller, D.M., 2002. Inbreeding effects in wild populations. *Trends in Ecology & Evolution* 17, 230-241.
- Klein, J., 1987. Origin of Major Histocompatibility Complex polymorphism - the transspecies hypothesis. *Human Immunology* 19, 155-162.
- Lampert, K.P., Bernal, X.E., Rand, A.S., Mueller, U.G., Ryan, M.J., 2006. No evidence for female mate choice based on genetic similarity in the tungara frog *Physalaemus pustulosus*. *Behavioral Ecology and Sociobiology* 59, 796-804.

- Landry, C., Bernatchez, L., 2001. Comparative analysis of population structure across environments and geographical scales at major histocompatibility complex and microsatellite loci in Atlantic salmon (*Salmo salar*). *Molecular Ecology* 10, 2525-2539.
- Lips, K.R., Diffendorfer, J., Mendelson, J.R., Sears, M.W., 2008. Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. *Plos Biology* 6, 441-454.
- Mainguy, J., Worley, K., Cote, S.D., Coltman, D.W., 2007. Low MHC DRB class II diversity in the mountain goat: past bottlenecks and possible role of pathogens and parasites. *Conservation Genetics* 8, 885-891.
- McCallum, H., Dobson, A., 2002. Disease, habitat fragmentation and conservation. *Proceedings of the Royal Society of London Series B-Biological Sciences* 269, 2041-2049.
- Mikko, S., Andersson, L., 1995. Low Major Histocompatibility Complex class-II diversity in European and North-American moose. *Proceedings of the National Academy of Sciences of the United States of America* 92, 4259-4263.
- Milinski, M., 2006. The major histocompatibility complex, sexual selection, and mate choice. *Annual Review of Ecology Evolution and Systematics* 37, 159-186.
- Miller, H.C., Lambert, D.M., 2004. Genetic drift outweighs balancing selection in shaping post-bottleneck major histocompatibility complex variation in New Zealand robins (*Petroicidae*). *Molecular Ecology* 13, 3709.
- Miller, K.M., Kaukinen, K.H., Beacham, T.D., Withler, R.E., 2001. Geographic heterogeneity in natural selection on an MHC locus in sockeye salmon. *Genetica* 111, 237-257.
- Nielsen, R., 2005. Molecular signatures of natural selection. *Annual Review of Genetics* 39, 197-218.
- Nikolich-Zugich, J., Fremont, D.H., Miley, M.J., Messaoudi, I., 2004. The role of mhc polymorphism in anti-microbial resistance. *Microbes and Infection* 6, 501-512.
- Nolet, B.A., Rosell, F., 1998. Comeback of the beaver *Castor fiber*: An overview of old and new conservation problems. *Biological Conservation* 83, 165-173.
- O'Brien, S.J., Evermann, F.F., 1988. Interactive influence of infectious disease and genetic diversity in natural populations. *Trends in Ecology and Evolution* 3, 254-259.
- O'Brien, S.J., Roelke, M.E., Marker, L., Newman, A., Winkler, C.A., Meltzer, D., Colly, L., Evermann, J.F., Bush, M., Wildt, D.E., 1985. Genetic basis for species vulnerability in the cheetah. *Science* 227, 1428-1434.
- Olsson, M., Madsen, T., Nordby, J., Wapstra, E., Ujvari, B., Wittsell, H., 2003. Major histocompatibility complex and mate choice in sand lizards. *Proceedings of the Royal Society of London Series B-Biological Sciences* 270, S254-S256.
- Paterson, S., Wilson, K., Pemberton, J.M., 1998. Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). *Proceedings of the National Academy of Sciences of the United States of America* 95, 3714-3719.
- Pearman, P.B., Garner, T.W.J., 2005. Susceptibility of Italian agile frog populations to an emerging strain of Ranavirus parallels population genetic diversity. *Ecology Letters* 8, 401-408.
- Penn, D.J., 2002. The scent of genetic compatibility: Sexual selection and the major histocompatibility complex. *Ethology* 108, 1-21.
- Peters, M.B., Turner, T.F., 2008. Genetic variation of the major histocompatibility complex (MHC class II beta gene) in the threatened Gila trout, *Oncorhynchus gilae gilae*. *Conservation Genetics* 9, 257-270.

- Piertney, S.B., Oliver, M.K., 2006. The evolutionary ecology of the major histocompatibility complex. *Heredity* 96, 7-21.
- Radwan, J., Kawalko, A., Wojcik, J.M., Babik, W., 2007. MHC-DRB3 variation in a free-living population of the European bison, *Bison bonasus*. *Molecular Ecology* 16, 531-540.
- Radwan, J., Tkacz, A., Kloch, A., 2008. MHC and preferences for male odour in the bank vole. *Ethology* 114, 827-833.
- Reid, J.M., Arcese, P., Keller, L.F., Elliott, K.H., Sampson, L., Hasselquist, D., 2007. Inbreeding effects on immune response in free-living song sparrows (*Melospiza melodia*). *Proceedings of the Royal Society B-Biological Sciences* 274, 697-706.
- Richman, A.D., Herrera, L.G., Nash, D., Schierup, M.H., 2003. Relative roles of mutation and recombination in generating allelic polymorphism at an MHC class II locus in *Peromyscus maniculatus*. *Genet Res* 82, 89-99.
- Ross-Gillespie, A., O'Riain, M.J., Keller, L.F., 2007. Viral epizootic reveals inbreeding depression in a habitually inbreeding mammal. *Evolution* 61, 2268-2273.
- Schwensow, N., Eberle, M., Sommer, S., 2008. Compatibility counts: MHC-associated mate choice in a wild promiscuous primate. *Proceedings of the Royal Society B-Biological Sciences* 275, 555-564.
- Schwensow, N., Fietz, J., Dausmann, K.H., Sommer, S., 2007. Neutral versus adaptive genetic variation in parasite resistance: importance of major histocompatibility complex supertypes in a free-ranging primate. *Heredity* 99, 265-277.
- Siddle, H.V., Kreiss, A., Eldridge, M.D.B., Noonan, E., Clarke, C.J., Pyecroft, S., Woods, G.M., Belov, K., 2007. Transmission of a fatal clonal tumor by biting occurs due to depleted MHC diversity in a threatened carnivorous marsupial. *Proceedings of the National Academy of Sciences of the United States of America* 104, 16221-16226.
- Smith, K.F., Acevedo-Whitehouse, K., Pedersen, A.B., 2009. The role of infectious diseases in biological conservation. *Animal Conservation* 12, 1-12.
- Smith, K.F., Sax, D.F., Lafferty, K.D., 2006. Evidence for the role of infectious disease in species extinction and endangerment. *Conservation Biology* 20, 1349-1357.
- Snell, G.D., 1968. The H-2 locus of the mouse: observations and speculations concerning its comparative genetics and its polymorphism. *Folia Biologica (Prague)* 14, 335-358.
- Sommer, S., 2005. The importance of immune gene variability (MHC) in evolutionary ecology and conservation. *Frontiers in Zoology* 2, 16.
- Spielman, D., Brook, B.W., Briscoe, D.A., Frankham, R., 2004. Does inbreeding and loss of genetic diversity decrease disease resistance? *Conservation Genetics* 5, 439-448.
- Takahata, N., Nei, M., 1990. Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of Major Histocompatibility Complex loci. *Genetics* 124, 967-978.
- van de Bildt, M.W.G., Kuiken, T., Visee, A.M., Lema, S., Fitzjohn, T.R., Osterhaus, A.D.M.E., 2002. Distemper outbreak and its effect on African wild dog conservation. *Emerging Infectious Diseases* 8, 211-213.
- van Oosterhout, C., Joyce, D.A., Cummings, S.M., Blais, J., Barson, N.J., Ramnarine, I.W., Mohammed, R.S., Persad, N., Cable, J., 2006. Balancing selection, random genetic drift, and genetic variation at the major histocompatibility complex in two wild populations of guppies (*Poecilia reticulata*). *Evolution* 60, 2562-2574.
- Weber, D.S., Stewart, B.S., Schienman, J., Lehman, N., 2004. Major histocompatibility complex variation at three class II loci in the northern elephant seal. *Molecular Ecology* 13, 711-718.

- Wegner, K.M., Kalbe, M., Reusch, T.B.H., 2007. Innate versus adaptive immunity in sticklebacks: evidence for trade-offs from a selection experiment. *Evolutionary Ecology* 21, 473-483.
- Whiteman, N.K., Matson, K.D., Bollmer, J.L., Parker, P.G., 2006. Disease ecology in the Galapagos Hawk (*Buteo galapagoensis*): host genetic diversity, parasite load and natural antibodies. *Proceedings of the Royal Society B-Biological Sciences* 273, 797-804.
- Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A., Losos, E., 1998. Quantifying threats to imperiled species in the United States. *Bioscience* 48, 607-615.
- Yamazaki, K., Boyse, E.A., Mike, V., Thaler, H.T., Mathieson, B.J., Abbott, J., Boyse, J., Zayas, Z.A., Thomas, L., 1976. Control of Mating Preferences in Mice by Genes in Major Histocompatibility Complex. *Journal of Experimental Medicine* 144, 1324-1335.
- Zhu, L., Ruan, X.D., Ge, Y.F., Wan, Q.H., Fang, S.G., 2007. Low major histocompatibility complex class II DQA diversity in the Giant Panda (*Ailuropoda melanoleuca*). *Bmc Genetics* 8, 29.

DRAFT

10. Appendices

Table 1. MHC and neutral variation in natural populations. The evidence for historical selection on MHC comes from a dN/dS ratio > 1 in the Antigen Binding Sites (ABS); Recent selection is inferred from deviation of genotype frequencies from expected Hardy–Weinberg proportions (H-W), lack of correlation of allelic richness with that for neutral alleles (correlation), lack of significant isolation by distance (IBD), higher or lower population differentiation than for neutral alleles (F_{ST} outlier, population differentiation F_{ST}), departures of allele frequency spectra from those expected under neutrality (Ewens-Watterson (E-W) and Slatkin’s P and BOTTLENECK tests for allele frequency data, Tajima’s D test for nucleotide sequence data).

Species (Taxon)	Evidence for departure from neutrality		Populations examined
	Historical	Recent (type of evidence)	
Teleosts			
Atlantic salmon (<i>Salmo salar</i>) ¹	YES(dN/dS)	YES (E-W) NO (H-W, correlation)	Comparison of land-locked and river populations
Brown trout (<i>Salmo trutta</i>) ²	YES(dN/dS)	NO (Tajima D, E-W, population differentiation (F_{ST}), correlation)	Small isolated populations
Brown trout (<i>Salmo trutta</i>) ³	Not estimated	YES (F_{ST} outlier significant (diversifying selection) for MHC linked microsatellite locus in large populations, in small populations effect masked by immigration)	Comparison of large and those that have declined in size
California coastal steelhead (<i>Oncorhynchus mykiss</i>) ⁴	YES(dN/dS)	NO (correlation, population differentiation F_{ST}), YES (F_{ST} outlier (diversifying selection) in one of 3 regions)	Populations that have experienced recent declines in size
Gila trout (<i>Oncorhynchus gilae gilae</i>) ⁵	YES(dN/dS)	NO (F_{ST} outlier)	Populations that have declined in size
Sockeye salmon (<i>Oncorhynchus nerka</i>) ⁶	YES(dN/dS)	YES (E-W (16% of pops), Slatkin’s P (9% of pops), population differentiation F_{ST})	31 river populations compared with one lake population
Amphibians			
Alpine newt (<i>Mesotriton alpestris</i>) ⁷	YES(dN/dS)	NO (correlation, F_{ST} outlier)	Groups of allopatric populations of postglacial origin
Crested newt (<i>Triturus cristatus</i>) ⁸	YES(dN/dS)	NO (correlation)	Comparison between refugial populations and populations from the postglacial expansion area
Birds			
Great snipe (<i>Gallinago media</i>) ⁹	YES(dN/dS)	YES (Tajima’s D, high structure between regions, not explained by neutral marker diversity, IBD)	Scandinavian mountain vs. East European population
Lesser kestrel (<i>Falco naumanni</i>) ¹⁰	YES(dN/dS)	YES (Tajima’s D) NO (correlation, IBD pattern)	Free ranging but fragmented wild populations
South island robin (<i>Petroica australis australis</i>) ¹¹	YES(dN/dS)	NO (correlation)	Small, bottlenecked population
Mammals			
Spotted suslik (<i>Spermophilus suslicus</i>) ¹²	YES(dN/dS)	NO (correlation, F_{ST} outlier)	Small, bottlenecked populations
Water vole (<i>Arvicola terrestris</i>) ¹³	YES(dN/dS)	NO (global F_{ST} outlier) YES (BOTTLENECK, higher diversity in MHC at the phase of low population density, stronger selection at DQA1 locus, at high density phase effect masked by migration)	Demographically fluctuating populations, comparison of low and high density phase

References: ¹(Landry and Bernatchez 2001) ²(Campos et al. 2006); ³(Hansen et al. 2007); ⁴(Aguilar and Garza 2006); ⁵(Peters and Turner 2008); ⁶(Miller et al. 2001); ⁷(Babik et al. 2008); ⁸ (Babik et al. 2009); ⁹(Ekblom et al. 2007); ¹⁰(Alcaide et al. 2008); ¹¹(Miller and Lambert 2004); ¹²Biedrzycka and Radwan 2008 ¹³(Bryja et al. 2007)

This is a draft review for consultation. Additional work is in progress. Please do not quote this document or any part therein without prior consent of the authors.

DRAFT