PROCEEDINGS OF THE ROYAL SOCIETY B

rspb.royalsocietypublishing.org

Comment

Cite this article: van Oosterhout C. 2013 Maintenance of major histocompatibility supertype variation in selfing vertebrate is no evidence for overdominant selection. Proc R Soc B 280: 20122501.
http://dx.doi.org/10.1098/rspb.2012.2501

Received: 19 October 2012
Accepted: 13 November 2012

Author for correspondence:
Cock van Oosterhout
e-mail: c.van-oosterhout@uea.ac.uk

The accompanying reply can be viewed at http://dx.doi.org/10.1098/rspb.2012.2854.

Royal Society Publishing
Informing the science of the future

Maintenance of major histocompatibility supertype variation in selfing vertebrate is no evidence for overdominant selection

Cock van Oosterhout

School of Environmental Science, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK

A principal challenge in evolutionary studies is that the evolutionary forces (i.e. mutation, recombination, gene flow, drift and selection) are rarely observed directly, but that they must be inferred from their effects on fitness, phenotype, behaviour, demography or genetic variation. Evolutionary biology therefore relies heavily on deductive logic, whereby premises are linked with conclusions. For example, one premise is that high parasite biodiversity will select for a genetically diverse immune system. Observing a positive correlation between parasite diversity and immunogenetic variation does not, however, imply causality, as there may be additional processes (e.g. population size and migration) that affect both of these variables. For instance, among primates, parasite species richness is lowest among the threatened species [1], and given that small population size and isolation will affect both genetic variation and parasite biodiversity, a spurious correlation may exist between both variables. For that reason, many population genetic studies on immune genes employ neutral genetic markers to account for the effects of population demography.

Ellison *et al.* [2] studied both microsatellite loci and major histocompatibility (MHC) immune genes, analysing how inbreeding affects the variation at both types of loci in the self-fertilizing fish, *Kryptolebias marmoratus*. In addition, they studied how pathogen resistance is affected by genetic variation, analysing the relative contribution of both microsatellite and MHC variation on parasite loads in natural and laboratory populations with different degrees of inbreeding. This is a strong experimental design, because measuring the loss in microsatellite variation over successive generations of selfing quantifies the effect of random genetic drift, and parasite load is directly linked to an individual's genotype.

Ellison *et al.* [2] found that both MHC and neutral variation were lost after several generations of selfing, an observation that is consistent with the effects of drift. However, MHC alleles were not lost at random, and, intriguingly, the most divergent alleles appeared to be maintained. In their analysis, they group alleles into MHC supertypes, which are defined based on the molecule binding properties of the positively selected sites. This is considered to be a good approximation of the functional differences [3], and given that the locus affiliation of MHC sequences was not known, this approach makes biological sense without losing too much information. The authors suggest that the maintenance of several divergent functional MHC supertypes in individuals with low microsatellite heterozygosity is indicative of overdominant selection. Given that individuals with a higher MHC diversity showed a lower parasite load, the authors hypothesize that overdominant selection could play a role in the maintenance of functional MHC diversity in *K. marmoratus*.

However, there is an alternative explanation for the maintenance of highly divergent MHC alleles that does not involve overdominant selection. The MHC is a multi-gene family often consisting of several duplicated class I and class II immune genes. These genes are generally highly polymorphic, and currently the paradigm is that (a combination of) parasite-mediated selection [4], pre- and post-copulatory sexual selection [5], and selection on the mutational load accumulated in the surrounding MHC region [6] are the selective forces

maintaining this MHC polymorphism. The conclusion, therefore, that the MHC polymorphism of *K. marmoratus* expressed in the number of MHC supertypes is maintained by parasite-mediated overdominant selection seems to fit the current paradigm. However, in this species, the authors observe that each fish possesses between 1 and 11 MHC alleles and between 1 and 8 supertypes. This suggests that some individuals have at least six MHC loci, which is consistent with a previous study of this fish that suggested there are 13 MHC class I loci [7]. Furthermore, Ellison *et al*. [2] report that no fish had more than two alleles of each supertype, which implies that alleles of distinct supertypes segregate at different loci. The evolutionary genetic implication of this is that even when alleles become fixed, supertype diversity will not be depleted because this variation is preserved across different loci. This could explain why, even after more than 10 generations of selfing, the average diversity of MHC supertypes per individual in the laboratory lines was similar to that found in the natural *K. marmoratus* population. Inbreeding can erode the within-locus variation when an alternative allele of the same supertype is lost. However, while inbred individuals possess fewer alleles, the remaining alleles are distinct from one another because they are from different supertypes. This explains why the observed mean amino acid difference is higher for (inbred) individuals with fewer MHC alleles (fig. 3 of [2]).

Even in the absence of overdominant selection, individuals can continue to possess multiple MHC supertypes in the form of a completely homozygous multi-locus genotype. This is what evidently happened in the R strain of Ellison *et al.*'s [2] study, in which all fish possessed the same genotype. The alternative explanation—that all selfing individuals are heterozygous for exactly the same alleles across all of their loci—simply defies the laws of probability. An additional problem with this explanation is that if all 13 unlinked class I MHC loci had to be kept heterozygous by overdominant selection, the segregation load would be so high that only one out of 8192 offspring (i.e. $\frac{1}{2}^{-13}$) produced by selfing would have a fitness (or immunocompetence) equal to that of the parental fish.

The presence of distinct MHC supertypes at different genetic loci would offer an important adaptive evolutionary advantage to a selfing species such as *K. marmoratus*, because vital immunogenetic variation will be preserved even in the face of the most severe inbreeding. This significantly reduces the costs of natural selection [8], and solves Haldane's Dilemma for selfing (and inbreeding-prone) species in which individuals rely on an immune system with multiple supertypes. Species that have not adapted to inbreeding may have the same allelic copy (or supertype) present at multiple loci, such as the guppy, *Poecilia reticulata*, in which individuals carried up to five almost identical MHC class II allele copies in their genome [9]. In such cases, inbreeding could exacerbate the loss of allelic variation by fixing the same copy across multiple loci, as was observed in the greater prairie chicken, *Tympanuchus cupido* [10].

From the valid premise that selfing *K. marmoratus* individuals have a genetically diverse immune system, one cannot logically conclude that this is maintained by overdominant selection. Conversely, the argument presented here does not discount the possibility that some form of balancing selection is operating on the MHC of these fish. In particular, balancing selection could act on haplotype variation and favour haplotypes with several diverse MHC supertypes. Indeed, this is consistent with the negative correlation between parasite load and the number of MHC supertypes. In other words, balancing selection is more likely to act on the variation across loci within haplotypes, rather than on the within-locus variation, which is prone to drift. So, while I disagree with Ellison *et al.*'s [2] conclusion that the study supports the hypothesis of immune-related overdominance in *K. marmoratus*, I concur that parasite-mediated balancing selection may play a role in this system. However, such selection is more likely to act on the variation present across rather than within MHC loci.

References

1. Altizer S, Nunn CL, Lindenfors P. 2007 Do threatened hosts have fewer parasites? A comparative study in primates. *J. Anim. Ecol.* **76**, 304–314. (doi:10.1111/j.1365-2656.2007.01214.x)
2. Ellison A, Allainguillaume J, Girdwood S, Pachebat J, Peat KM, Wright P, Consuegra S. 2012 Maintaining functional major histocompatibility complex diversity under inbreeding: the case of a selfing vertebrate. *Proc. R. Soc. B* **279**, 5004–5013. (doi:10.1098/rspb.2012.1929)
3. Naugler C, Liwski R. 2008 An evolutionary approach to major histocompatibility diversity based on allele supertypes. *Med. Hypotheses* **70**, 933–937. (doi:10.1016/j.mehy.2007.09.015)
4. Spurgin LG, Richardson DS. 2010 How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. *Proc. R. Soc. B* **277**, 979–988. (doi:10.1098/rspb.2009.2084)
5. Edwards SV, Hedrick PW. 1998 Evolution and ecology of MHC molecules: from genomics to sexual selection. *Trends Ecol. Evol.* **13**, 305–311. (doi:10.1016/S0169-5347(98)01416-5)
6. van Oosterhout C. 2009 A new theory of MHC evolution: beyond selection on the immune genes. *Proc. R. Soc. B* **276**, 657–665. (doi:10.1098/rspb.2008.1299)
7. Sato A, Satta Y, Figueroa F, Mayer WE, Zaleska-Rutczynska Z, Toyosawa S, Travis J, Klein J. 2002 Persistence of Mhc heterozygosity in homozygous clonal killifish, *Rivulus marmoratus*: implications for the origin of hermaphroditism. *Genetics* **162,** 1791–1803.
8. Haldane JBS. 1957 The cost of natural selection. *J. Genet.* **55**, 511–524. (doi:10.1007/BF02984069)
9. Llaurens V, McMullan M, van Oosterhout C. 2012 Cryptic MHC polymorphism revealed but not explained by selection on the class IIB peptide-binding region. *Mol. Biol. Evol.* **29**, 1631–1644. (doi:10.1093/molbev/mss012)
10. Eimes JA, Bollmer JL, Whittingham LA, Johnson JA, van Oosterhout C, Dunn PO. 2011 Rapid loss of MHC class II variation in a bottlenecked population is explained by drift and loss of copy number variation. *J. Evol. Biol.* **24**, 1847–1856. (doi:10.1111/j.1420-9101.2011.02311.x)