

The Role of Immunogenetics in Amphibian Susceptibility to Chytridiomycosis

Batrachochytrium dendrobatidis (Bd) causes chytridiomycosis in the world's amphibians. Bd infects the skin leading to respiratory issues, nervous system shutdown, and death. The fungus is the main driver of amphibian extinction, exceeding the extinction rate of all vertebrates (Fisher & Garner, 2020).

Aims & Objectives:

I propose to research whether MHC class II β alleles (Bataille et al., 2015) which confer resistance to chytridiomycosis are present in many amphibian species using sequence analysis of the gene in infected susceptible and resistant species globally. If Bd resistant alleles are highly expressed in resistant species and susceptible who survive infection, this could indicate a positive selection pressure driving that allele expression to protect against chytridiomycosis.

To preserve biodiversity and prevent species loss, it is important to know factors which increase resistance to chytridiomycosis. The reintroduction of amphibian species which have the resistant MHC allele could prevent mortality, depending on the results of this research.

Literature review:

The major histocompatibility complex (MHC) class II proteins in amphibians obtain protein fragments, antigens, from Bd during infection for display on the cell surface as T cell receptors.

The MHC plays a role in amphibian resistance to chytridiomycosis (Savage & Zamudio, 2011). Certain MHC class II (MHC-II) β alleles, lead to a conformational change in the MHC class II binding groove, altering affinity to Bd antigens (Bataille et al., 2015).

The identification of MHC providing resistance to chytridiomycosis and the MHC genotypes responsible for resistance provide the basis of my research and allows the extrapolation that certain MHC alleles confer Bd resistance.

Methodology:

Research will use quantitative computer-based methods. Therefore, there is a reduction in limitations of travel restrictions, social distancing, and self-isolation. No Garda vetting or ethics approval is required.

Resources:

This project will rely on computational tools such as R studio, the Basic Local Alignment Searching Tool (BLAST) program and the Genbank sequence database, which are freely available from a computer.

There is potential to develop my research, requiring funding, during a scheduled two-week research expedition to Honduras, under the research organisation Operation Wallacea. I have a research assistant role in Cusuco National park, Honduras, investigating chytridiomycosis prevalence in endemic amphibian species using polymerase chain reaction (PCR) to examine Bd DNA sequences. There may be an increasing number of species susceptible or resistant to Bd, therefore I could

include those sequences in my research. The more species involved, the less likely correlations are due to chance or only applicable to one genus. Due to Covid-19, it is uncertain whether the expedition will continue to go ahead.

Role allocation:

I will act as principal investigator of the research with all aspects being my responsibility. This research is an opportunity to learn valuable analysis skills by conducting a sequence analysis of amphibian species and applying statistical testing in practice.

Dr Aoife McLysaght, will act as an advisor and provide a collaborative experience with an academic for me as an undergraduate. The role will involve progress meetings and review of work to ensure it is robust, plus discussion of sequence analysis techniques and statistical methodology to use based on my hypothesis.

Timeline:

Weeks 1&2:

Sourcing MHC-II β gene sequence data for 50 susceptible and 50 resistant amphibian species on the Genbank database.

Accession Number (Genbank)	Species Data	Research Paper
JN638850–JN638882	Population genetic background (PBR) of 33 alleles of an expressed MHC Class II β gene of Bd infected frogs.	Savage, A. E., & Zamudio, K. R. (2011). MHC genotypes associate with resistance to a frog-killing fungus. <i>Proceedings of the National Academy of Sciences</i> , 108(40), 16705–16710. https://doi.org/10.1073/pnas.1106893108
KJ679288–KJ679331	MHC-II β sequence from 17 global amphibian species with varying susceptibility to Bd. MHC genotyping data of <i>Litoria verreauxii alpina</i> wild populations from Dryad Digital Repository	Bataille, A., Cashins, S. D., Grogan, L., Skerratt, L. F., Hunter, D., McFadden, M., ... Waldman, B. (2015). Susceptibility of amphibians to chytridiomycosis is associated with MHC class II conformation. <i>Proceedings of the Royal Society B: Biological Sciences</i> , 282(1805), 20143127. https://doi.org/10.1098/rspb.2014.3127
MF555153–MF555185	9,11, and 13 MHC-II β sequences from <i>R. japonica</i> , <i>R. ornativentris</i> and <i>R. t. tagoi</i> , respectively.	Lau, Q., Igawa, T., Minei, R., Kosch, T. A., & Satta, Y. (2017). Transcriptome analyses of immune tissues from three Japanese frogs (genus <i>Rana</i>) reveals their utility in characterizing major histocompatibility complex class II. <i>BMC Genomics</i> , 18(1). https://doi.org/10.1186/s12864-017-4404-0
HQ025929–HQ025945, BC092157, D50039, EF210753, and NM_001114771	12 exon 2 MHC-II β sequences from 7 ranid species. MHC sequences from <i>X. laevis</i> species.	Kiemnec-Tyburczy, K. M., Richmond, J. Q., Savage, A. E., & Zamudio, K. R. (2010). Selection, trans-species polymorphism, and locus identification of major histocompatibility complex class II β alleles of New World ranid frogs. <i>Immunogenetics</i> , 62(11-12), 741–751. https://doi.org/10.1007/s00251-010-0476-6

Table 1. Accession numbers and associated literature for MHC-II β genes in various amphibian species

Weeks 3&4:

BLAST searches performed using a DNA/protein sequence of a particular MHC-II β allele from an amphibian species (Table 1) as a Query. The BLAST search of a particular amphibian’s MHC-II gene sequence used to find MHC-II β sequences in other amphibians. If the species’ susceptibility to Bd is known, the resistance-level of that allele can be inferred.

The search, using a specific MHC-II β allele sequence, can locate the same allele (homologous) in other amphibian species, therefore susceptibility of that species is known.

Weeks 5&6:

Conduct a two-sample Kolmogorov-Smirnov test using R studio. Sample size of 50 recovered susceptible amphibian species and 50 resistant species used to compare distributions. If a significant result is found in 100 species, it aids result extrapolation to the world's amphibian population. The null hypothesis states the MHC-II β allele of an amphibian does not affect Bd resistance. The alternative hypothesis is the allele does affect resistance.

If the alternative hypothesis fails to be rejected, the significance (p -value < 0.01) that certain alleles increase resistance to Bd infection will be investigated using a chi-squared test in R studio. If the result is highly significant, there is evidence for positive selection acting upon Bd resistant MHC-II β alleles in amphibian populations.

Data visualisation constructing relevant graphs and tables based on statistical testing and analysis of results. Conclusion formed from the results of statistical analyses.

Potential limitations:

A species heterozygous for the Bd resistant MHC-II β gene may encode an MHC that can bind more antigens than homozygous with two susceptible alleles (Hughes, 1989). Species previously proven resistant will be described as such based on resistant allele presence in the homozygous or heterozygous condition, since there is no evidence to refute resistant alleles displaying heterozygous dominance.

Certain MHC-II β alleles may confer resistance in a minority of species, therefore the results of analysis of 100 species may be incorrect in its conclusion that there is no relationship between MHC-II β allele and disease resistance. Previous literature states a relationship between MHC-II conformation and susceptibility to Bd infection in 17 global amphibian species (Bataille et al., 2015), thus it is unlikely to be the case.

The inexperience of performing sequence analysis and statistical testing may result in misreading result significance. The inclusion of my supervisor will reduce this limitation by reviewing work to ensure the correct information is included.

Collaboration:

Based on the global nature of the chytridiomycosis pandemic and my association with Operation Wallacea, I hope to collaborate with researchers involved in chytridiomycosis studies to find more DNA sequences for susceptible/resistant amphibian species. I will present my findings to the organisation's researchers in the hopes it will aid amphibian conservation efforts in Honduras and globally.
