



GENETIC CHARACTERIZATION, GEOGRAPHICAL VARIATION AND INHERITANCE OF MHC CLASS IIB IN *Bombina pachypus*

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The Major Histocompatibility Complex (MHC) is a gene-dense region directly involved in the adaptive immune response, and variation is usually high in MHC loci. MHC polymorphism is believed to be maintained by pathogen-driven selection, mediated through balancing selection (i.e. heterozygote advantage or frequency-dependent selection). However, the knowledge about this system is poor, particularly in amphibians. Furthermore, amphibians typically show both high susceptibility to pathogenic infections and genetically differentiated population, often due to reduced gene flow.

Therefore, this PhD project aimed to

- I) characterize MHC class IIB loci in the endangered Apennine yellow-bellied toad (*Bombina pachypus*), an Italian endemic amphibian;
- II) assess the potential role of selection in shaping MHC geographic pattern in a natural context in which genetic drift may act as not negligible evolutionary force;
- III) investigate MHC inheritance in a small interspecific hybrid population of *Bombina* in Italy.

We genotyped over than 280 individuals from 42 sampling sites in the entire distribution range of the species. NGS amplicon sequencing (Illumina MiSeq technology) allowed to amplify all exon2 variants of MHC class IIB and a specific pipeline was set up to validate genotypes. We found overall 32 well-differentiated DNA variants encoding 30 putative functional amino acid sequences. The analysis of the sequences alignment showed signals of positive selection acting in the past and the phylogenetic network revealed a few cases of Trans-Species Polymorphisms (i.e. the occurrence of similar alleles in congeneric species). Basically, MHC diversity across populations was distributed according to the pattern “southern richness and northern purity” and appeared to be related to neutral variation (previously assessed by 9 microsatellites loci). These results lead concluding that MHC variation is mainly shaped by genetic drift in the natural populations of the Apennine yellow-bellied toad, despite its adaptive significance. Analysis of MHC inheritance in the hybrid population is still in progress.