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Ph. D. THESIS

«Molecular analysis of Major Histocompatibility Complex (MHC) in natural and breeding populations of animal species»

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ABSTRACT

The importance of adaptive genetic polymorphisms is a fundamental and unsolved issue in biology. Improved understanding of the relative contribution of evolutionary forces requires knowledge of interactions between genetic polymorphisms and adaptation. Major histocompatibility complex (MHC) genes are among the most polymorphic loci in nuclear genome of mammals, and their function is well studied at the molecular level. MHC is a multigene receptor family that binds and presents antigenic peptides to T-cells. Diversity in these genes indicates that they are selected. Hence their study helps in understanding the role of natural selection in the shaping of genetic polymorphisms and is considered as a possible model for testing the hypothesis for the reasons and consequences of selection. The study of natural populations of non-model species with cosmopolitan distribution could contribute in this direction. In this study intra- and inter-population genetic polymorphisms within the second exon of *DQA* and *DRB1* loci in MHC have been examined in hares (*Lepus europaeus*), wild boars (*Sus scrofa scrofa*), domestic pigs (*Sus scrofa* L. *domestica*) and sheeps (*Ovis aries*). These two loci are among the most polymorphic in MHC class II genes. For this reason, were performed a complete population genetic analysis of both genes in all three species. PCR-SSCP was used to definethe *DRB1* and *DQA* alleles in each species, followed by identification of their sequences. Results were analyzed *in silico* with bioinformatics methods. In each species *DRB1* was more polymorphic than *DQA*. These two loci are under the influence of balancing selection, indicated by excess of non-synonymous substitutions ($d_N/d_S>1$). The data suggest frequency-dependent selection as a major mechanism. In L. europaeus there is remarkable genetic polymorphism in MHC with Anatolia exhibiting the greatest levels. According to the post-glaciation distribution pattern of hare, North-European populations should hold lower numbers of polymorphisms compared to South refugia (Balkan, Anatolia) as has been shown by mitochondrial DNA analysis. However, European populations appeared to be highly polymorphic at NHC, attributed to balancing selection. Conversely, *S. scrofa* exhibits reduced polymorphisms and that is partly explained both from bottleneck effects in recent wild boar past and from pig domestication. In parallel, has to be disentangled the role of Balkan Peninsula in pig domestication and in shaping boar diversity. Finally, both genes exhibit increased polymorphism in *O. aries* and the reasons have to be attributed to pathogens,

geographic or social factors and environmental heterogeneity. The data indicate that MHC cannot be used in phylogeography studies as gene alleles show weak phylogeographic signal. Trans-species evolution of MHC is pervasive in all phylogenetic trees. Binding pockets are possible to respond to selection forces in a way that depends on their role in peptide binding whereas in both genes there are charged amino acid substitutions with common residues in all three species. The correlation of different alleles with disease resistance or susceptibility is of great practical importance because markers for genetic selection could be developed for individuals and races, with important applications in pig and sheep breeding.