“The Role of Hybridization in the Evolutionary Response to Environmental Change in the Genus *Canis*”

Roya Adavoudi Jolfaei, M. Sc

This thesis investigates hybridization patterns among *Canis* species, including free-ranging dogs (*Canis lupus familiaris*), Eurasian gray wolves (*Canis lupus*), and golden jackals (*Canis aureus*), across Eurasia. The genome-wide single nucleotide polymorphisms (SNP) data was applied to explore the ecological and evolutionary factors shaping hybridization patterns among these species. While hybridization between domestic dogs and grey wolves has been previously studied, including the golden jackal provides new insights into the effect of the evolutionary distance between the cross-breeding species on hybridization and introgression rates.

This thesis presents a comprehensive analysis of hybridization within the genus *Canis*, aiming to achieve a deeper understanding of how hybridization operates not only as a source of interspecific gene flow but also as a mechanism for local adaptation and evolutionary change. For this purpose, the thesis addresses five key objectives: (1) investigate possible consequences of hybridization and its impact on the parental species, (2) find the best method for estimating proportions of hybridization-derived variants in gray wolves, golden jackals, and free-ranging dogs, (3) estimate the rate of hybridization between the studied taxa using this method, (4) assess the role of hybridization in species adaptation by identifying adaptive introgressed chromosomal fragments and assessing the functions of genes included in these fragments, (5) test the effect of environmental variables on the proportions of hybridization-derived variants and adaptive introgression rates in canid populations. These objectives are addressed in four chapters of the thesis.

Chapter 1 presents a systematic literature review of studies that have reported the consequences of hybridization across various mammalian orders and families. Our results showed that negative consequences of hybridization, like genetic swamping (reported in 21% of studies) and introgression of variants from domestic animals (reported in 18% of studies), have been reported in the literature more frequently compared to the positive consequences, like gaining novel adaptive variation (reported in 8% of studies). The predominance of negative outcomes reported in the literature can be explained by the fact that many studies are based on neutral genetic markers, which are limited in detecting complex processes like adaptive introgression or hybrid speciation. Therefore, integrating both neutral loci and markers located in coding regions can provide a more comprehensive and balanced understanding of hybridization, capturing not only its potential negative consequences but also its adaptive benefits and the underlying factors that shape these outcomes.

Chapter 2 addressed the second objective by evaluating the methods of individual ancestry reconstruction at the global (i.e. in the entire genomes) and local levels (within chromosomes) in the context of the analysis of introgressive hybridization among the three canids, based on genome-wide SNP data. The results revealed that global ancestry methods (e.g., PCA and ADMIXTURE) generally estimated higher hybridisation levels than local ancestry methods (e.g., LAMP-LD, ELAI, and Ghap). The inconsistency between the results may result from differences in their methodological frameworks, the types of genetic information they utilize, and their strategies for handling missing data. Two key factors, low-quality genotypes and subpopulation structure, were identified as major factors that can contribute to increasing uncertainty and variability between methods. We found that global ancestry analyses such as ADMIXTURE are more likely to be affected by these confounding factors. Therefore, we recommend a joint use of local and global methods, with results of local ancestry analysis being prioritized for precise estimation of introgression levels.

Chapter 3 addressed the third and fourth objectives by exploring the evolutionary consequences of hybridization in canids. Based on the methodological knowledge from the last chapter and the robust performance of ELAI in estimating individual ancestry proportions, the introgression rate among species was estimated using ELAI. Our results showed that hybridization in the genus *Canis* is common in their distribution range. In some regions, including the Balkans, India, the Caucasus, and northeastern Europe, a higher frequency of hybridization was found, which may have resulted from high anthropogenic disturbances, large population size of free-ranging dogs, and the range expansion of golden jackals. We also clearly show the effect of evolutionary distances between the species on introgression rates between them, since a higher frequency of dog introgression was found in wolves compared to golden jackals (6.4% vs. 1.2%). The evolutionary consequences of hybridization showed that both wild canids and free-ranging dogs may gain benefits from hybridization. Adaptive introgression may enable wild canids to acquire from dogs gene variants conferring adaptive advantage, including those that strengthen their immune systems. These beneficial genes may increase the resistance of wild canids to new pathogens, which would be particularly beneficial in environments where wild canids encounter dogs frequently and where pathogens are constantly evolving. Free-ranging dogs appear to have acquired a larger pool of beneficial genetic variants from wolves, which may have contributed to some characteristics like morphological, behavioural, and physiological traits. Alongside detecting signals of positive selection, we also found signatures of negative selection in chromosomal blocks with reduced introgression levels in dogs and golden jackals. These results suggest that some introgressed gene variants may also have a deleterious effect on these species, but they can be efficiently removed from their gene pools. Overall, we highlight the complex nature of hybridization and introgression in the evolutionary process, showing that it can introduce both beneficial and maladaptive genetic variation.

Chapter 4 addresses the last objective by performing the Random Forest (RF) analysis and Redundancy analysis (RDA) to identify the key environmental factors that may contribute to the frequency of dog-derived genetic variants in wild canids. In wolves, the frequency of such variants showed positive association with milder winters, while in golden jackals, a reverse trend was observed. Regions with milder winters likely support larger free-ranging dog populations, increasing the likelihood of contact and interbreeding between dogs and wolves. Conversely, in golden jackals, lower annual temperatures may drive individuals toward human settlements in search of food, increasing interactions with domestic dogs and thereby hybridization rates. Additionally, our results showed that frequency of dog-derived genetic variants in wild canids is positively correlated with human footprint. Regions with high human disturbance often have a greater abundance of free-ranging dogs, increasing the likelihood of encounters with wild canids. Furthermore, we found a significant association between dog-derived loci under adaptive introgression in wolves and environmental factors. The genes under adaptive introgression were associated with the nervous system, immune system, and metabolism. These results emphasize the role of adaptive introgression, which can help wolves to better adapt to human-modified environments, where wolves may encounter new pathogens, environmental stressors, and dietary shifts. We highlighted the role of hybridization as an active evolutionary process, possibly being more important for adaptation than previously believed.

Overall, this thesis presents a comprehensive investigation into the ecological drivers and evolutionary consequences of hybridization between wild canids and free-ranging dogs. Using an integrative approach that combined genomic analyses, selection scans, and environmental associations, we achieved a deeper understanding of how hybridization operates not only as a source of interspecific gene flow but also as a mechanism for local adaptation. The results of this study highlight the complex nature of hybridization and introgression in the evolutionary process. While variants under adaptive introgression may enhance local adaptation of species, hybridisation may also introduce deleterious variants, potentially disrupting locally adapted gene complexes or increasing vulnerability to disease and other stressors. The results of this study have practical implications for wildlife management and conservation. Recognizing when hybridization contributes to adaptive potential and when it threatens species integrity will be critical for informed decision-making in conservation genetics, particularly for taxa that include domesticated lineages, such as the genus *Canis*.