INSTYTUT OCHRONY PRZYRODY

POLSKIEJ AKADEMII NAUK

Dr hab. Aleksandra Biedrzycka
al. Adama Mickiewicza 33, 31-120 Kraków
tel. 12 370-35-00
e-mail: sekretariat@iop.krakow.pl

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Review of the doctoral dissertation by Ms. Francelly Martinez Sosa entitled:
"The effect of environmental factors on adaptive genetic variation in grey wolves (Canis lupus) and free-ranging dogs (Canis lupus familiaris)"
conducted at the Museum and Institute of Zoology of the Polish Academy of Sciences under the supervision of Dr. hab. Małgorzata Pilot

Ms. Francelly Martinez Sosa obtained her Master's degree in 2019 from the University of Lincoln (United Kingdom). As part of her Master's thesis, she studied the conservation genetics of *Macaca sylvanus* (Thesis title: *Conservation genetics of Algerian Barbary macaques (Macaca sylvanus)*). Earlier stages of her education were completed at the University of Puerto Rico. At that institution, in the years 2012–2015, she was also employed in several research projects as a research assistant, where she managed laboratory work and was involved in student education and supervision. She also worked at the Caribbean Primate Research Center. She has not previously applied for a doctoral degree.

1. Scientific value of the dissertation

a. Originality of the research

In the doctoral dissertation submitted for evaluation, Ms. Martinez Sosa describes the adaptive evolution of two closely related taxa—grey wolves and free-ranging dogs—by analyzing a large part of their range and using an extensive set of SNP loci (single nucleotide polymorphisms). The study of adaptive changes at the genomic level is currently a widely applied approach that allows researchers to address fundamental evolutionary questions. More importantly, identifying genetic variation in key genomic regions that potentially enables survival under changing environmental conditions allows to predict shifts in species ranges and assess adaptive potential in response to climate change.

The comparison of these two taxa—whose ranges largely overlap but which differ in ecological niches and food resource use—provides excellent material for studying molecular adaptations and for addressing how different environmental conditions shape adaptive changes over relatively short evolutionary timescales. The Author demonstrates that both in wolves and free-ranging dogs, signatures of selection are present in the same genes associated with essential traits, such as olfaction and morphological characteristics. However, these genes exhibit signatures of divergent selection, suggesting that different trait values are favoured in the two taxa.

Analyses of natural selection patterns in relation to local environmental characteristics revealed different genes under selection in each taxon. The Author explains this either by a potentially strong influence of demographic factors on genetic variation in wolf populations or by distinct genetic mechanisms facilitating adaptation to local conditions in the two taxa.

Determining the relationship between genetic variation and environmental conditions allowed to estimate the adaptive potential of domestic dogs and to identify natural habitats providing optimal

survival conditions. She applied an innovative approach, genomic offset (GO) analysis, which demonstrated that free-ranging dogs, particularly those from southern Eurasia, are likely to have higher than wolves survival potential under a warming climate. The high genetic variability of these populations, together with environmental modelling analyses, also indicates that this region may have been the site of the original domestication of dogs.

Taken together, the results provide a comprehensive picture of molecular evolution and adaptation in response to environmental conditions in both taxa and, importantly, allow predictions about future range dynamics. The dissertation undoubtedly presents an original and modern solution to a scientific problem. The Author demonstrates broad theoretical knowledge and, above all, an excellent methodological skillset that enables insight into the adaptive history of two species of exceptional ecological and social importance.

b. Scientific value of the chapters/articles

The research results are presented in four chapters. The first has already been published as a review article in the journal *Genes* (MDPI group), while the remaining three have not yet been published. The dissertation is extensive and contains comprehensive and detailed results, which I discuss further below.

Although I have no major reservations regarding the applied methodology or the conclusions drawn, I find that the dissertation lacks an overarching synthesis and broader conclusions that would allow for more general statements about adaptive processes and their significance in the context of climate change. These issues are mentioned but not fully developed. As the Author states, her goal was to conduct comparative genomic analyses to identify patterns of genetic variation resulting from adaptive and demographic processes. Indeed, the dissertation is largely descriptive in character. The focus on detailed presentation of results is also reflected in the rather concise treatment of the study hypotheses, which appear more fitted to the obtained results than formulated as specific research questions grounded in existing knowledge.

The most compelling part of the dissertation, in my opinion, is the final chapter, which aims to estimate adaptive potential in the context of climate change and to identify the region of dog domestication. Here, the application of advanced methodological approaches allowed the Author to address clearly formulated hypotheses, and the discussion highlights the practical implications of the findings.

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2. Evaluation of the articles included in the dissertation

The first article is a review summarizing research published between 2016 and 2022 on molecular mechanisms underlying adaptive evolution in vertebrates in response to environmental changes. The review indicates that adaptive evolution in vertebrates most often involves regulatory elements, gene expression, and cellular pathways. An interesting outcome is the identification of gene loss as a mechanism facilitating adaptation to local conditions. Although gene loss or the reduction of entire gene families has previously been suggested in adaptive contexts, the increasing confirmation of this phenomenon in recent genomic studies constitutes an important contribution to understanding adaptive processes at the species level.

While the topic is important for expanding knowledge about adaptive variation beyond single nucleotide changes, I have some reservations regarding the substantive level of the manuscript. I miss references to earlier comprehensive reviews summarizing mechanisms of adaptive evolution and a clearer statement of what novel contribution this review provides. It would also have been valuable to present adaptive mechanisms in relation to different environmental conditions. The

manuscript provides a detailed description of published cases. I also have reservations regarding its structure, as information is sometimes repeated in both tables and text, and summary figures synthesizing the findings would have been helpful. Moreover, this review is only loosely connected to the subsequent analytical chapters and is rarely referenced in the general description of the dissertation.

The three subsequent chapters use complementary methods and present coherent results. It should be emphasized that the research design was ambitious both in terms of sampling area and analytical scope. According to the statements provided, Ms. Martinez Sosa played a significant role in the presented research.

Chapters 2 and 3 focus on analyses of genetic structure, patterns of divergent selection in populations of both taxa, and identification of loci associated with environmental conditions. The dataset is extensive, covering most of the Eurasian range of both taxa, and analyses are based on an initial set of 700,000 SNP loci. The selection of analytical approaches for estimating interpopulation differentiation, identifying outlier loci and gene groups under selection, and detecting loci associated with environmental variables was comprehensive, appropriate, and correctly conducted.

However, both chapters lack a clear and detailed grounding in the theoretical framework of adaptive evolution and do not present clearly formulated, specific hypotheses. The theoretical introduction to Chapter 2 concludes with a general statement that the aim was to explain how natural selection acts on two closely related canid taxa with overlapping ranges but different ecological niches. This objective is broadly formulated, and the chapter does not fully explain the similarities and differences in selection acting on the two species but rather presents descriptions of individual polymorphisms identified as outliers. The discussion partially repeats the results and lacks generalized conclusions. I particularly appreciate, however, the discussion of variation in the gene associated with canine distemper virus, where possible causes of divergent selection between populations are thoughtfully examined.

The relationship between interpopulation structure analyses and selection analyses is not clearly articulated. I do not question the necessity of population structure analyses as background for selection studies, but the reader is left without a proposed and subsequently resolved cause-and-effect framework.

The introduction to Chapter 3, examining associations between genetic variation and environmental characteristics, presents detailed aims and predictions, though again not explicitly formulated as research hypotheses. In the methods, it would be helpful to explain why species richness was used as an environmental variable. It would also be worth addressing the fact that, for dogs, half as many loci were included in the analyses compared to wolves. Section 4.4, summarizing shared evolutionary patterns in both taxa, seems particularly important for drawing general conclusions but is treated rather briefly.

I appreciate the discussion linking specific loci to ecological and climatic indicators. The Author clearly and insightfully explains associations with drought levels detected in wolves and associations with overall species richness in habitats detected in free-ranging dogs, whereas in wolves such a relationship was observed only for ungulate species richness.

The final chapter analyzes adaptive potential, defined as the level of genetic variation enabling survival under changing environmental conditions in free-ranging domestic dogs. Previously identified SNP loci associated with local habitat characteristics were used, and environmental niche modeling was applied to predict potential range shifts under projected climate change scenarios. Combining these approaches enabled the estimation of genomic offset, i.e., the discrepancy between

the current composition of variation at loci under local selection and the composition that may be required under future climate scenarios.

Once again, the Doctoral Candidate demonstrates remarkable proficiency in bioinformatic analyses, enabling the application of a highly innovative approach to address the approximate location of dog domestication and predict future distribution changes under climate warming. This chapter best articulates the research aims and predictions. The genomic offset analyses and niche modeling allow for accessible graphical presentation of results, which are discussed logically and comprehensively. The Author demonstrates the ability to critically interpret her findings and draw synthetic conclusions.

Despite the critical remarks above, I assess the scientific quality of the dissertation very highly, as further reflected in my final evaluation.

3. Editorial and formal correctness

The main chapters are preceded by abstracts in Polish and English. Unfortunately, the Polish summary appears to be a raw machine translation and contains serious linguistic errors, including incorrect grammatical agreement. Although I do not expect the Author to know Polish, in a doctoral dissertation it would be advisable to have the Polish summary proofread by a native speaker.

The final section summarizing the research synthesizes the three analytical chapters but does not refer to the initial review article. I also miss a clearer presentation of explicit links and a cause-and-effect narrative connecting all four chapters.

5. Final evaluation

I read the doctoral dissertation of Ms. Francelly Martinez Sosa with great interest. The Author used a very extensive dataset and applied well-conceived research methods. She demonstrated exceptional proficiency in bioinformatic and statistical analyses, enabling the generation of detailed and comprehensive results that provide insight into the adaptive evolution of the grey wolf and domestic dog.

Before publication of the research findings, it would be advisable to focus on clearly formulating research hypotheses and explicitly referring to them in the discussion, which would allow the full potential of these highly valuable results to be realized.

Despite the above-mentioned remarks, I conclude that the doctoral dissertation of Ms.Francelly Martinez Sosa meets all the requirements for doctoral theses and I unequivocally evaluate it positively.

I hereby state that the reviewed doctoral dissertation of Ms. Francelly Martinez Sosa fulfils the conditions specified in Article 187 of the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended), and I submit a motion to the Council of the Museum and Institute of Zoology of the Polish Academy of Sciences in Warsaw to admit Ms. Francelly Martinez Sosa to the subsequent stages of the doctoral proceedings.

Signature Not Verified

Dokument podpisany przez
Aleksandra Biedrzycka
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