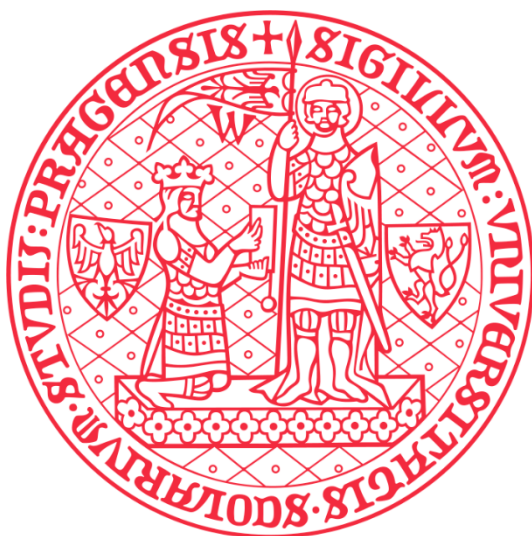


**CHARLES UNIVERSITY
FACULTY OF SCIENCE**

Study programme: Zoology



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Adaptive population shifts in response to climate change
Adaptivní změny rozšíření populací v odpovědi na klimatické změny

Doctoral thesis

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Praha, 2020

Prohlašuji, že jsem dizertační práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce, ani její podstatná část nebyla předložena k získání jiného, ani stejného akademického titulu.

V Praze, dne 18. 11. 2020



Ing. Michaela Horníková

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Acknowledgements

First and foremost I would like to thank to Petr Kotlík for his patient supervision and guidance during my doctoral studies. I am very grateful for the stimulating environment with the right amount of challenges he provided me, for always letting me address those challenges myself and for sharing his knowledge and insight whenever I got lost. I also appreciate his support for my passion to learn new skills and I am especially thankful for all the opportunities for presenting the results of my research on both national and international conferences. My thanks also go to Silvia Marková for her co-supervision, especially for her immense help with all the laboratory methods and subsequent analyses, for all the discussions over the results, for her invaluable help with manuscript writing and also for creating nice and friendly environment in the office. I also want to thank to Marco Escalante for his invaluable help, especially for sharing his knowledge on ENM and for always being friendly and enthusiastic. I also appreciate that my research would not be possible without the work of our laboratory technicians and many other awesome people in Liběchov and also without our collaborators in the USA who provided invaluable insight during the phase of manuscript writing.

I wish to express many thanks to my family for all the emotional and material support I got throughout the years. The biggest appreciation goes undoubtedly to my mother and my husband - I am very grateful for all the motivation, patience, tolerance, love and kindness that they showed me, as well as for all the lunches, dinners, chocolates, ice cream sundaes and bottles of wine and beer I got as a form of support from them.

I am also grateful to my family and to all my friends for showing interest in my work and for pretending convincingly they really understood when I tried to explain what the heck am I doing and why is it so interesting.

The research included in this thesis was supported by the grants to the Laboratory of Molecular Ecology of the Institute of Animal Physiology and Genetics CAS, from the Czech Science Foundation (grant numbers: 16-03248S and 20-11058S) and the Ministry of Education, Youth and Sports of the Czech Republic (projects: KONTAKT II LH15255 and EXCELLENCE CZ.02.1.01/0.0/0.0/15_003/0000460 OP RDE).

Summary

Understanding of species' reactions to past climate and environmental changes is a hot topic in many fields of biology as it is relevant also for addressing species' future under the contemporary climate change. Using an emerging model species, the bank vole, I combine genomic phylogeographic data with information on known intraspecific functional variability and environmental niche modelling and aim to elucidate the particular role of intraspecific variation and ultimately selection in shaping the species' response to the climatic and environmental changes after the end of the last glaciation. Based on the mtDNA markers, bank voles exhibit a complex phylogeographic pattern suggesting population replacement events during the postglacial recolonization of Europe and thus possible involvement of selection in the process. An extensive dataset of more than 6000 SNPs was used to search for signs of population replacement in the bank vole genomic DNA and to investigate the species' postglacial recolonization history throughout its European distribution range. The genomic data revealed even more complex population history than previously detected with mtDNA markers, including not only admixture but also population replacement between intraspecific lineages originating in different glacial refugia. Gene flow between locally adapted populations could enable exchange of alleles beneficial under particular climatic conditions, influencing which populations would be successful in colonizing particular areas. A convincing example of possible local adaptation of the bank vole intraspecific lineages - functional variation in haemoglobin coding genes - had been previously identified. Results presented here show that the geographical distribution of the particular beneficial allele responsible for variation in Cys-based antioxidant capacity of red blood cells is a result of not only population history, but it is also influenced by environmental conditions within the bank vole distribution range, suggesting involvement of selection in shaping the distribution. As the results further suggest, the duplicate haemoglobin genes of the bank vole could represent an example of gene-regulatory genetic variation interacting with a well-defined protein-coding variation to control a functional trait, maintaining the polymorphism in populations and fine tuning the phenotype in response to the selection pressure. Ecological niche modelling further supports the existence and importance of possible adaptive intraspecific divergence showing that on the intraspecific level, there are significant differences in ecological niches of the bank vole populations originating in different refugia. In general, the results presented here not only demonstrate importance of SNP phylogeography and ecological niche modelling on the intraspecific level in the research of species' postglacial history, but most importantly show that different intraspecific evolutionary lineages might play differently important role in shaping species' reaction to climate change or even for its survival.

Shrnutí

Porozumění tomu, jak druhy reagovaly na změny klimatu a prostředí v minulosti, je žhavým tématem v mnoha oborech biologie, jelikož je relevantní i pro řešení otázky budoucnosti druhů pod vlivem současných klimatických změn. S využitím modelového druhu norníka rudého propojují genomická fylogeografická data s informacemi o známé vnitrodruhové funkční variabilitě a environmentálním modelováním nik a zaměřují se na objasnění konkrétní úlohy vnitrodruhové variability a selekce v utváření odpovědi tohoto druhu na klimatické a environmentální změny po skončení posledního zalednění. Norník rudý vykazuje podle mtDNA markerů komplexní fylogeografický pattern, naznačující nahrazení populací během postglaciální kolonizace Evropy a tedy možný vliv selekce v tomto procesu. K pátrání po známkách nahrazení populací v genomické DNA norníka rudého a k prozkoumání postglaciální rekolonizační historie v rámci jeho evropského areálu rozšíření jsme použili rozsáhlý datový soubor s více než 6000 SNP. Genomická data odhalila ještě složitější populační historii, než byla dříve detekována pomocí mtDNA, zahrnující nejen nahrazení populací, ale také příměs mezi vnitrodruhovými liniemi pocházejícími z různých glaciálních refugií. Tok genů mezi lokálně adaptovanými populacemi by mohl umožnit výměnu alel výhodných za konkrétních klimatických podmínek a ovlivnit tak, které populace by byly úspěšné v kolonizaci konkrétních oblastí. U norníka rudého byl již dříve popsán přesvědčivý příklad možné lokální adaptace vnitrodruhových linií – funkční variabilita genů kódujících hemoglobin. Výsledky prezentované zde ukazují, že geografické rozšíření výhodné alely zodpovědné za variabilitu v antioxidační kapacitě červených krvinek je výsledkem nejen populační historie, ale že je také ovlivněno podmínkami prostředí v rámci areálu norníka rudého, což naznačuje možný vliv selekce. Jak výsledky dále naznačují, duplikované geny kódující hemoglobin by mohly představovat příklad interakce mezi genově regulační variabilitou a jasně definovanou variabilitou v protein kódujících genech kontrolující funkční znak, udržující polymorfismus v populacích a doladující fenotyp v odpovědi na selekční tlak. Modelování ekologických nik dále podporuje existenci a význam možné adaptivní vnitrodruhové divergence tím, že ukazuje, že na vnitrodruhové úrovni existují signifikantní rozdíly v ekologických ních populací norníka rudého pocházejících z různých refugií. Obecně zde prezentované výsledky ukazují nejen význam SNP fylogeografie a modelování ekologických nik na vnitrodruhové úrovni pro studium postglaciální historie druhů, ale především poukazují na to, že různé vnitrodruhové linie mohou hrát různě významnou roli pro utváření reakce daného druhu na klimatické změny nebo dokonce pro jeho přežití.

List of publications included in the thesis

- 1) Marková, S., **Horníková, M.**, Lanier, H. C., Henttonen, H., Searle, J. B., Weider, L. J., Kotlík, P. (2020). High genomic diversity in the bank vole at the northern apex of a range expansion: the role of multiple colonizations and end-glacial refugia. *Molecular Ecology*, 29: 1730– 1744. <https://doi.org/10.1111/mec.15427>
- 2) **Horníková, M.**, Marková, S., Lanier, H. C., Searle, J. B., Kotlík, P. (2020). Not just endemism hotspots: A dynamic history of admixture from Mediterranean and extra-Mediterranean glacial refugia drives high genomic diversity in the bank vole. In preparation for submission to *Molecular Ecology*.
- 3) **Strážnická, M.**, Marková, S., Searle, J. B., & Kotlík, P. (2018). Playing hide-and-seek in beta-globin genes: gene conversion transferring a beneficial mutation between differentially expressed gene duplicates. *Genes*, 9(10), 492. <https://doi.org/10.3390/genes9100492>
- 4) Dvořáková, V., **Horníková, M.**, Němcová, L., Marková, S., Kotlík, P. (2020). Regulatory variation in functionally polymorphic globin genes of the bank vole: a possible role for adaptation. *Frontiers in Ecology and Evolution*, 7, 514. <https://doi.org/10.3389/fevo.2019.00514>
- 5) Escalante, M., **Horníková, M.**, Marková, S., Kotlík, P. (2020). Niche divergence in a postglacial colonizer, the bank vole *Clethrionomys glareolus*. Submitted to *Journal of Biogeography*, under revision.

Introduction

The contemporary anthropogenic warming of the climate system, unprecedented over the decades and millennia, poses a challenge to both human and natural systems (IPCC, 2014). It has already negatively impacted many species in at least part of their range (Pacifiçi *et al.*, 2017) and biodiversity on all levels from organism to whole biomes is anticipated to be further affected (Bellard *et al.*, 2012; Nunez *et al.*, 2019). This in turn will inevitably impact not only the ecosystems and their functions but also the human well-being and the dynamics of the climate change itself (Pecl *et al.*, 2017). Understanding and ultimately predicting how organisms react to the climate change is vital for informing biologically sound and effective conservation strategies and mitigation policies (Scheffers and Pecl, 2019) and therefore it became a hot topic in many fields of biology.

To persist under the new climatic conditions, e.g. different mean and more extreme temperatures, species need to change (either through plastic or genetic response) along one or more of the three axes of geographical space, time and ‘self’ (Bellard *et al.*, 2012). Examples of such responses like shifts in range, phenology or physiology and also signatures of evolutionary genetic adaptations have been observed in many terrestrial and marine species (reviewed in e.g. Parmesan, 2006; Bertin, 2008; Chen *et al.*, 2011; Peñuelas *et al.*, 2013; Poloczanska *et al.*, 2013; Scheffers *et al.*, 2016), however, our understanding of relative importance of these responses as species climate change coping mechanisms is still considerably limited as is also our ability to account for all important non-climatic factors that influence and shape these responses (Pacifiçi *et al.*, 2015; Foden *et al.*, 2019). Contemporary intraspecific genetic structure can provide invaluable insights into the history of the species and past processes that shaped that structure, including neutral and adaptive genetic changes in response to the changes in the climate that occurred in the past. In case of European fauna and flora, the most significant driver inducing demographic changes and providing opportunities for adaptations to occur were the Quaternary climatic oscillations and therefore their ‘genetic legacy’, i.e. their impact on species’ genetic structure, offer a unique opportunity to study the mechanisms of species’ response to climate change (Hewitt, 2000). It was proposed that it has been the interplay of migration and adaptation and not either process alone that has shaped the species’ response to the Quaternary climate change (Davis and Shaw, 2001). Despite some evidence of selective processes during the Quaternary leaving an imprint in species’ current genetic diversity (Mayol *et al.*, 2015), assessing relevance of natural selection for the species’ response to the past climatic change is still challenging.

The extent to which the evolutionary adaptation from novel mutations could help mitigate the effect of climate change is considered to be limited in comparison to range shift or plastic response (Parmesan, 2006; Gienapp *et al.*, 2008), however, the potential role of existing intraspecific variation in the species range shift dynamics should not be underestimated. The environmental heterogeneity over particular species distribution range can promote local adaptations, leaving some individuals better suited for particular conditions than others. Temporal environmental variation also induces variability in selection pressure over time and therefore even conditionally neutral intraspecific genetic variation both within and between populations might become a substrate for selection driven by the climate change at some point, either facilitating the species’ rapid evolutionary response to changing environmental conditions *in situ* or during colonization of novel environments (Kovach *et al.*, 2012; Henry and Russello, 2013; Bay and Palumbi, 2014; Madsen *et al.*, 2020; Capblancq *et al.*, 2020). Success and extent of the species range shifts therefore might be

determined not only by the presence or absence of geographical barriers and suitable habitat but also by their populations' genetic makeup.

Methodological framework

An excellent framework to gain more insight into the relationship between the species' range shifts and its genetic constitution can be found in phylogeography, a discipline that examines relationships between genetically distinct intraspecific lineages and interprets them in geographical context focusing on which processes lead to current geographical distribution of those lineages (Avice *et al.*, 1987). The favourite marker of the early years of phylogeography, the universal and presumably selectively neutral mtDNA, is nowadays often complemented (or replaced) by various nuclear genomic markers. The precipitous technological advances in the last decade have brought fast and relatively low-cost sequencing technologies and approaches such as genotyping-by-sequencing (GBS), which make it possible to obtain large single nucleotide polymorphism (SNP) datasets from targeted fractions of the genome even for species with no previous genomic information and large genomes. The routine availability of large number of genomic markers also for non-model species makes it possible to test and refine previously proposed historical scenarios and fully elaborate the idea that the range shifts during the recolonization of Europe after the Last Glacial Maximum (LGM) might not have been a selectively neutral process after all, first brought up by numerous evidence of complete or partial population replacements during the recolonization process derived predominantly from the mtDNA (Barnes *et al.*, 2002; Hofreiter *et al.*, 2007; Searle *et al.*, 2009). This idea, challenging then popular view of recolonization as a rather simple process of southern populations spreading northwards into vacant areas along available colonization routes, was further corroborated also by the evidence of replacements taking place under the contemporary climate change (Cook, 1975). The population replacements very convincingly pointed to a possibility that selection acting upon genetic differences between the populations was involved in shaping their current distribution. Consequently, a new sub-discipline of phylogeography was born - adaptive phylogeography. Building on the original framework, it tries to determine which parts of the populations' genetic makeup might have been favoured by selection in the recolonization process (Kotlík *et al.*, 2014). The cornerstone of adaptive phylogeography is an integrative approach combining phylogeographic data with the assessment of possible adaptive value of intraspecific genetic variation. This was exemplified in the study of Kotlík and colleagues (2014) who determined the genetic basis of the investigated phylogeographic pattern of a gene with known functional importance and inferred the functional phenotypic differences between the genetic variants involved. Such combination of top-bottom and bottom-up approaches can help to pinpoint genetic variants with presumed physiological importance and answer the question whether or not these variants were under selection pressure posed by changing climatic conditions at the end of the last glaciation (Barrett and Hoekstra, 2011). Even though the top-bottom approach requires phenotypic data which is unavailable when addressing species' response to the past climate change, it can be used to identify candidate genes or genetic regions underlying phenotypic traits of known functional importance or traits that vary with environmental factors in extant populations (Atwell *et al.*, 2010; Igoshin *et al.*, 2019). When a polymorphism with known physiological outcome is identified, its distribution and evolutionary history can be investigated using population and landscape genetics methods to infer possible signs of past environmental selection. Genetic-environment association can be inferred by correlating the

distribution of functionally important genetic variants with environmental variables. Various methods enabling fast environmental association inference while controlling for neutral genetic structure are now available (Rellstab *et al.*, 2015). Accounting for the neutral genetic structure is important to disentangle the signatures of demographic history versus local adaptation because despite being result of neutral mechanisms such as genetic drift, the neutral population structure can mimic patterns generated by non-neutral processes (Excoffier *et al.*, 2009). Available methods differ by their approach to address this issue, performance and computational requirements and thus by their applicability to large scale genetic and / or environmental datasets (Rellstab *et al.*, 2015). A logistic regression based spatial analysis method implemented in Samβada software represents a powerful option suitable for both assessment of environmental association of genetic variants with known functional importance and *de novo* identification of putatively adaptive loci. Samβada offers a multivariate analysis to assess effect of more than one explanatory environmental variable and thus makes possible to control for neutral population structure by including a variable to represent it. It can also assess possible relatedness between individuals by calculating spatial autocorrelation for the loci of interest, i.e. by evaluating whether similar genotypes tend to cluster in space (Stucki *et al.*, 2017). Such information related to particular functionally important genetic variants can be further complemented by applying the bottom-up methods within population and landscape genomics framework to thousands of genotyped loci in order to identify other regions within the species' genome showing signatures of adaptive variation and to link these with environmental variation and evolutionary processes (Rellstab *et al.*, 2015).

Specific historical scenarios regarding species' response to past climatic changes that may be formulated based on the integration of the environmental associations with population and landscape genetics and genomics data within the framework of phylogeography can be tested by rigorous statistical methods, such as approximate Bayesian computation (ABC). ABC provides a framework for testing hypotheses about species' demographic history, such as postglacial recolonization history (Tison *et al.*, 2014; Queirós *et al.*, 2019), including putatively adaptive population replacements in response to climate change (Kotlík *et al.*, 2018) and other complex demographic history patterns (Hudson *et al.*, 2020). Instructive implementations of ABC include also studies inferring invasion history (Lombaert *et al.*, 2014; Fraimout *et al.*, 2017) or ecological speciation (Momigliano *et al.*, 2017). ABC enables quantitative comparison of alternative historical scenarios of population divergence and admixture to find the one that could best explain the observed genetic variation across populations. It does so by comparing datasets simulated from prior distribution under the hypothesized evolutionary scenarios and summarized as vectors of summary statistics to the observed data (Cornuet *et al.*, 2008; Bertorelle *et al.*, 2010). Computational cost of simulating large enough reference tables makes the most common method of scenario choice on the basis of approximated posterior probabilities rather difficult for large SNP datasets, but with the emergence and implementation of new machine-learning approaches, the so called 'random forest' (RF), this problem can be overcome for both crucial steps of the ABC framework - the model choice (Pudlo *et al.*, 2015) and parameter estimation (Raynal *et al.*, 2018). By aggregating information from large number of binary decision trees trained on random subsamples of the reference table, i.e. the simulated data, the RF not only lowers the computational efforts needed but also offers more discrimination power and robustness than earlier ABC solutions, allowing to deal with bigger datasets and more complex models (Pudlo *et al.*, 2015; Kotlík *et al.*, 2018; Raynal *et al.*, 2018).

To properly address the role of demographic and adaptive processes in species' response to the Quaternary climate dynamics, both the spatial and temporal components of the species'

evolutionary dynamics need to be addressed. While ABC enables elucidation of the species' demographic history and population dynamics, the spatial component of the species' dynamics with its environment can be inferred by ecological niche modelling (ENM), a correlative approach based on the so called realized niche of the species (Hutchinson, 1957). Relying on the assumption that species' current distribution represents reasonably well its environmental (namely climatic) requirements, it uses correlation of the observed distribution with climatic variables to infer occupied ecological niches of particular species and to estimate areas with suitable conditions for that species. Hence, niche modelling can be used for hindcasting past distributions to investigate the role of spatial as well as temporal environmental heterogeneity in determining a species' demographic history (Fløjgaard *et al.*, 2009). By coupling the ENM with demographic and genetic data, temporally and spatially dynamic models to investigate species' response to past (Wachter *et al.*, 2016; Nicolas *et al.*, 2017) or even contemporary and future climatic changes can be created. (e.g. Rojas-Soto *et al.*, 2012; Razgour *et al.*, 2018; Ruaro *et al.*, 2019). It is increasingly being appreciated that to increase the biological realism of the derived models, it is necessary to account for various non-climatic factors influencing species' ability to track their environmental optimum, such as habitat loss and fragmentation (Watson and Segan, 2013; Segan *et al.*, 2015), dispersal (Williams and Blois, 2018), or competition (Urban *et al.*, 2012; Marion and Bergerot, 2018). Incorporating evolutionary adaptation (or the capacity thereof) into the ENM has also proved to have major impact on the resulting predictions (Bush *et al.*, 2016; Peterson *et al.*, 2019; Razgour *et al.*, 2019). The common practice for most ENM studies, however, is to construct the models on the level of the whole species, i.e. to assume that all populations within the species share the same ecological niche (e.g. Fløjgaard *et al.*, 2009; Vega *et al.*, 2010; Jacinto-Padilla *et al.*, 2017). Many species don't fit this assumption of ecological and evolutionary uniformity across their range - spatially heterogeneous environment coupled with reduced gene flow between species' populations encourages local adaptations of intraspecific lineages, leading also to niche divergence between them (Smith *et al.*, 2019). For example, the isolation of species in glacial refugia during the last glacial maximum (LGM) promoted not only intraspecific genetic divergence (Hewitt, 1996, 2000), but also may have facilitated local adaptations to different environmental conditions encountered in the geographically distinct refugia. Therefore, coupling the ENM approach for hindcasting the species distribution in the past with the information on intraspecific genetic variation across the species range to estimate past habitat suitability on population level could help us to understand the influence of environmental heterogeneity on the intraspecific niche divergence and its role in species' response to climate change, such as during the end-glacial colonization. Understanding how the intraspecific genetic variation, local adaptations and resulting niche divergence between the evolutionary lineages shaped the species range shifts in response to the past climate change is ultimately relevant also for the future predictions.

Model species

Following the integrative framework and approaches outlined above, I combine genomic phylogeographic data, information on distribution, evolutionary history and environmental association of intraspecific functional variability, and ecological niche modelling to elucidate the role of intraspecific genetic and ecological variation and selection in response of the bank vole (*Clethrionomys glareolus* (Schreber, 1780); aka *Myodes glareolus*, Fig. 1) to the climatic and environmental changes. This small muroid rodent from subfamily *Arvicolinae* is an emerging model species for

various studies, including of experimental evolution (Konczal *et al.*, 2015, 2016), physiology (Bonda-Ostaszewska *et al.*, 2019), population ecology (Haapakoski and Ylönen, 2010) and epidemiology (Voutilainen *et al.*, 2016; Khalil *et al.*, 2019). It is a typical representative of temperate forest-dwelling species with wide Palearctic distribution and can be found in all kinds of woodlands, scrub and parklands and hedges. Its range stretches from Mediterranean in the south to within the Arctic Circle in the north and from westernmost Europe to the Altai Mountains in Siberia (Fig. 1). Such distribution, together with short reproductive cycle and high number of offspring which allows for faster emergence of novel genetic variants compared to k-selected species, makes the bank vole an ideal model species for studies of postglacial colonization of Europe in the context of possible intraspecific adaptive divergence.

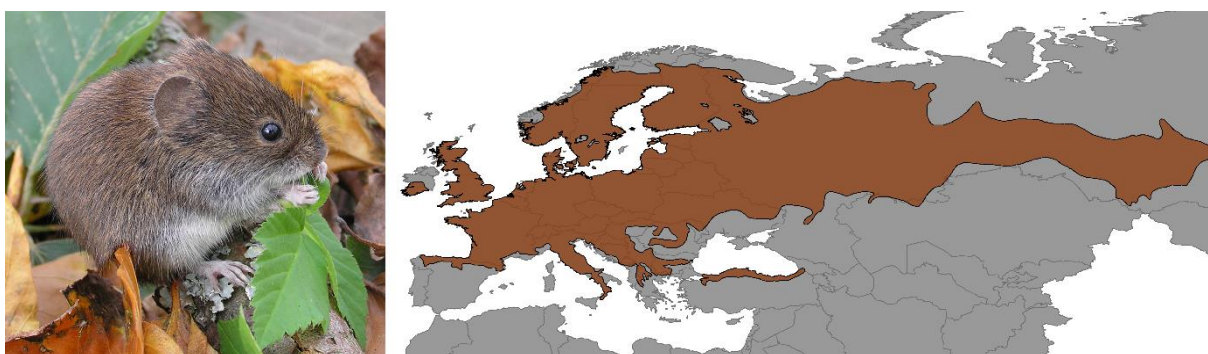


Fig. 1 Photo of the model organism, the bank vole (left, courtesy of Petr Kotlík) in its natural woodland habitat and map of its distribution range (right).

The majority of the early bank vole phylogeographic studies utilized then highly popular cytochrome *b* (*cob*) gene sequence of mtDNA as a marker (Bilton *et al.*, 1998; Deffontaine *et al.*, 2005). Discovery of a distinct mtDNA lineage in the area of the Carpathian Mountains with signatures of significant gene flow out of the Carpathians (Kotlík *et al.*, 2006) strongly supported the at that time still quite revolutionary hypothesis of the so called ‘cryptic northern refugia’, proposed by Stewart and Lister (2001). Supported also by fossil data (Sommer and Nadachowski, 2006), the Carpathian lineage provided evidence that not all species survived the harsh unfavourable conditions of the LGM solely in southern refugia in the Mediterranean peninsulas, as was the prevailing hypothesis at that time (Taberlet *et al.*, 1998).

Bank voles, together with several other species of small mammals, also played their part in challenging the neutrality of phylogeographic patterns. In Great Britain, two distinct bank vole mtDNA lineages with geographically delimited distribution were discovered, pattern that pointed out to a two phase colonization of Great Britain with a replacement event (Searle *et al.*, 2009). It was proposed that the genotype currently found in the peripheral areas of northern, western and southern Britain is a relic of the first colonizing population that entered Britain during the brief temperate period of the late glacial (i.e. before Younger Dryas (YD) cold spell), that was later (after the YD but before the submergence of the land bridge to Britain) partially replaced by a second colonizing population, currently occupying central and eastern parts of Britain (Searle *et al.*, 2009). Similar pattern pointing to the population replacement was found in the mtDNA and karyotype characteristics of several other small mammal species (Piertney *et al.*, 2005; Searle *et al.*, 2009) and the abrupt climatic changes at the end of the LGM were proposed as a key factor in those replacement events (Searle *et al.*, 2009). This result was later corroborated in the bank vole by genomic analysis of an extensive nuclear SNP dataset which not only validated the two phase

colonization scenario by using approximate Bayesian computation, but also showed that the replacement of the first colonists in southern Britain was genome-wide (Kotlík *et al.*, 2018). Linking the populations in northern periphery to the Carpathian mtDNA clade and the central population to the Western mtDNA clade (Filipi *et al.*, 2015) also supported the hypothesis, showing that both colonists originated in latitudinally separate refugia in continental Europe – Carpathian lineage originated in more northerly located refugium in the vicinity of the Carpathian Mountains, whereas the Western lineage presumably had its refugium in the foothills of the Alps (Kotlík *et al.*, 2006). Consequently, it was hypothesized that different ecological conditions in those refugia might have resulted in differences in genetic constitution of the two colonizing populations and that these differences could have given one of them an adaptive advantage over the other, promoting the population replacement (Searle *et al.*, 2009; Filipi *et al.*, 2015). No evidence was found for the replacement being promoted by functional divergence in the sequences of mtDNA genes encoding proteins (Filipi *et al.*, 2015), however the pattern of geographically delimited distribution of the two colonists was found to be mirrored in a physiologically important nuclear gene.

Two distinct haemoglobin variants differing in charge and consequently also in their migration speed during gel electrophoresis were discovered in British bank voles and named as HbS (slow) and HbF (fast) (Hall, 1979). A recent molecular genetic analysis of the genetic basis of the two variants revealed presence of a single non-synonymous amino acid substitution in both paralogous beta-globin coding genes, HBB-T1 (major) and HBB-T2 (minor), which differ in expression level (Kotlík *et al.*, 2014). Kotlík *et al.* (2014) also showed that the two variants represent geographically partitioned functional variation, as serine (Ser) in codon 52 of HbS is replaced by cysteine (Cys) in HbF. Cys contains a thiol group (-SH) that can, under appropriate molecular conditions, become a highly reactive functional group (Jacob *et al.*, 2005). It easily ionizes into a thiolate anion which can form disulphide bonds in an oxidation reaction with other thiol groups. If the thiol group is exposed on the surface of a protein molecule, physiologically important intermolecular disulphide bonds can be formed with thiols of other proteins and non-protein molecules (Reischl *et al.*, 2007). Haemoglobin containing such exposed thiol group can function as highly functional intracellular antioxidant capable of both regenerating the pool of the important non-enzymatic antioxidant glutathione (GSH) and intercepting reactive oxygen species directly (Rossi *et al.*, 1998). The Cys present in the bank vole beta-globin is indeed a completely exposed Cys residue and as it was demonstrated using the TRAP test (total radical-trapping antioxidant potential), the 52Cys haemoglobin variant significantly increases erythrocyte resistance to a free-radical attack compared to the 52Ser variant (Kotlík *et al.*, 2014). Energetically demanding physiological states and also thermal stress markedly increase the rate of reactive oxygen species (ROS) production (Pörtner, 2002) and therefore the 52Cys haemoglobin variant would likely be advantageous under various ecological conditions (Losdat *et al.*, 2014). Since the 52Cys variant is present predominantly in southern Britain, in populations of the second colonist, it was hypothesized (Kotlík *et al.*, 2014) that its distribution could be a result of natural selection by geographical variation in environmentally induced oxidative stress. This hypothesis is concordant with that of Hall (1979), who initially proposed environmental selection as a possible factor shaping the north-south distribution of the HbS and HbF variants.

Since the discontinuous distribution of some mtDNA lineages, namely the Carpathian and Eastern, suggests that genetic replacement events did not take place only in Great Britain, but also in Europe, namely southern Scandinavia and presumably also western Europe (Fig. 2), similarly detailed research of population shifts and replacement on the larger geographical scale is crucial to determine whether the conclusions from Great Britain can be generalized to other geographic

areas. Namely whether there is evidence of genome-wide population replacement events in continental Europe and whether they could have been driven by selection, involving populations possibly adapted to different local conditions they encountered in distinct glacial refugia (Kotlík *et al.*, 2014, 2018). This is of particular relevance as populations shifting their range in response to the current climate change will inevitably interact with populations already present in the surrounding areas, which may result in population replacement as well as in population's failure to shift its range (Atkins and Travis, 2010). Understanding how the distribution of particular populations was affected by their genetic constitution during the postglacial recolonization and uncovering the mechanisms behind the population replacements that took place in that period can ultimately help us to make more accurate predictions of species' response to current and future climate change.

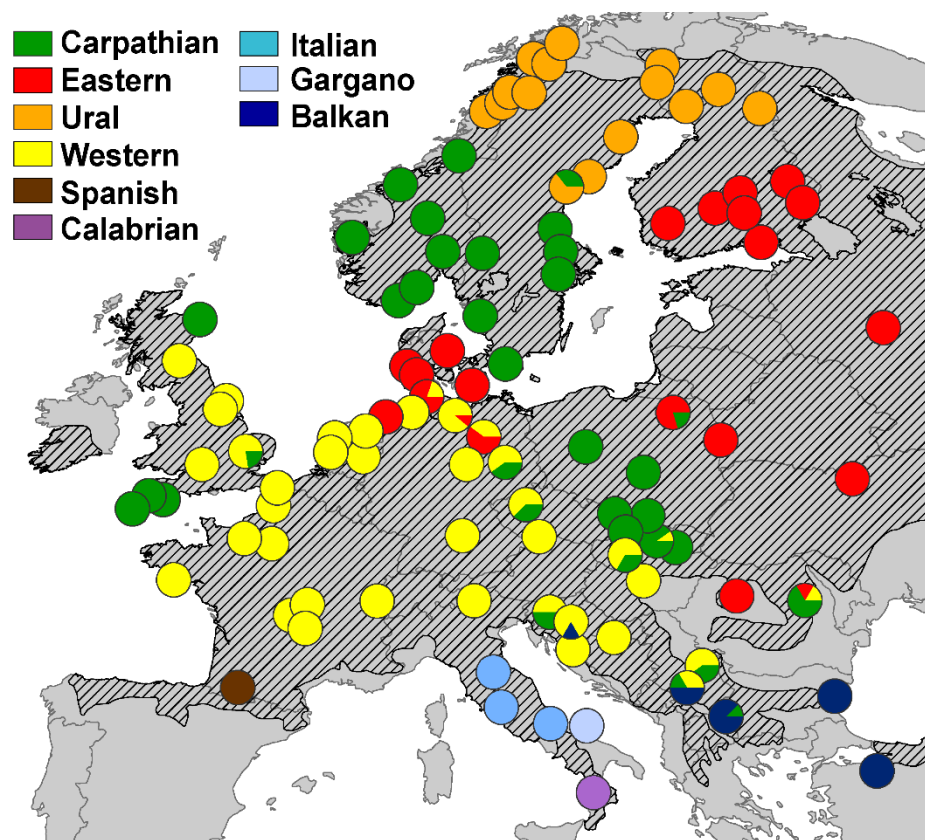


Fig. 2 Map of the distribution of the main bank vole mtDNA clades showing the discontinuous distribution of some clades, namely the Carpathian and Eastern, created based on the data from Marková, Horníková *et al.* (2020).

Outline of the thesis

Building on the results of previous research (e.g. Kotlík *et al.*, 2006, 2014, 2018; Searle *et al.*, 2009; Wójcik *et al.*, 2010; Marková *et al.*, 2014; Filipi *et al.*, 2015) I assess the role of intraspecific variation between different refugial populations and environmental selection in the process of recolonization of Europe by the bank vole. In particular, I address: **1)** whether the replacement events suggested in Europe by the mtDNA are reflected also in the nuclear genome of the continental populations of the bank vole and if yes, **2)** whether the functional variability in haemoglobin could have contributed to adaptive differences between populations involved in those events and **3)** whether there are any ecological differences between the populations taking part in the replacement events.

Paper 1 and **Paper 2** provide a detailed picture of the bank vole population history within its European range based on genomic data. The discontinuous distribution of some bank vole mtDNA lineages suggested that multiple events of population replacement had taken place during the recolonization of Europe by the small mammal. While in Great Britain this replacement proved to be true genome-wide replacement (Kotlík *et al.*, 2018), the population history in continental Europe where the British populations originated was still unresolved. Therefore, over 800 samples from more than 100 localities spanning the whole bank vole European distribution range were selected to include representative populations from all major refugial lineages (as approximated by their mtDNA). Using genotyping by sequencing (GBS), an extensive dataset of more than 6000 SNPs was built. Population structure was described using both maximum likelihood and spatially explicit Bayesian methods, individuals were assigned into genomic clusters and evolutionary relationships between those clusters were analysed using maximum likelihood method, which can account for admixture in the tree building process. These results were then used to estimate possible colonization history of bank voles in Europe by comparison of plausible scenarios using approximate Bayesian computation.

In **Paper 1** results with a focus on northern apex of the bank vole end-glacial expansion, Scandinavia, are presented. Scandinavia, similarly as Great Britain, is on the periphery of the bank vole distribution and the gene flow to the area through a major colonization route was interrupted several times and finally stopped completely by submerging of the land bridge connecting Scandinavia to Europe via Denmark. Consequently, hypotheses about colonization routes are easier to define and test than in the central Europe. Support for the hypothesis of bi-directional colonization of Scandinavia previously formulated based on the mtDNA is presented and role of northern refugia (i.e. Carpathian Mountains) and multiple colonization waves of populations derived from these refugia in postglacial recolonization of northern Europe and shaping genetic diversity patterns in that area is discussed. The results demonstrate how complementing the mtDNA with SNP phylogeography provides a detailed insight into demographic history and can reveal different or more complex patterns. This is corroborated by presented results from Denmark, where the anticipated population replacement suggested by mtDNA is reflected also in SNPs, but the nuclear markers further show signs of admixture from a third ancestral population. Furthermore, the SNP phylogeography reveals signs of admixture from Eastern lineage (via Denmark) also in populations from southern Sweden previously undetected by mtDNA.

Paper 2 presents the results of investigation of the colonization history of bank voles in central continental Europe, where the situation is much more complex than in Great Britain and Scandinavia, as the gene flow was not hindered by submersion of the key parts of colonization

routes. Building on the premise corroborated by the results from Paper 1 that due to the presumed population replacement the mtDNA and SNP phylogeography can show different histories, I evaluate and compare historical scenarios formulated based on the data from both markers. The roles of the Mediterranean and extra-Mediterranean (i.e. ‘cryptic northern’) refugia in colonization of this part of Europe are discussed, completing the picture delineated by the studies focused on Great Britain (Kotlík *et al.*, 2018) and Scandinavia (Marková, Horníková *et al.* 2020).

Paper 1 was published in *Molecular Ecology* (Marková, Horníková *et al.*, 2020), Paper 2 is presented in a form of a manuscript in preparation for submission to a journal *Molecular Ecology* (Horníková *et al.*, 2020).

In **Paper 3**, I focus on the possible role of the functional variation in the haemoglobin coding genes in shaping the contemporary distribution of the bank vole populations in Europe. In the first part of the paper, the distribution of the functional variability in haemoglobin coding genes is described for bank voles in continental Europe, where the variants discovered in Great Britain originated (Kotlík *et al.*, 2014). These results are derived from more than 500 samples from over 70 sites covering the whole species range within Europe. Then the possible role of variability in environmental conditions and population structure in shaping that distribution is evaluated. Correlation between the 52Cys beneficial allele distribution and several environmental variables was revealed by two correlation methods using 19 temperature and rainfall variables extracted for each site in ArcGIS. This result suggests possible involvement of environmental selection in shaping the 52Cys allele distribution. However, non-random association between the 52Cys allele at the nuclear locus and the Western mtDNA lineage was also found. The importance of both the historically determined population structure and environmental selection driven by the climate change in shaping the contemporary intraspecific functional variability distribution is therefore discussed. The second part is focused more on the evolutionary aspect of the variation. The beneficial 52Cys allele segregates in both major and minor haemoglobin genes that differ in expression level (Kotlík *et al.*, 2014). The haemoglobin coding genes exhibit signs of bidirectional gene conversion spanning the 52Cys codon and so I discuss the possible role of the gene conversion as a mechanism enabling tuning of erythrocyte thiol levels by reshuffling genotypes between the high- and low expressed paralog and thus as a mechanism of physiological adaptation of populations to new or changing environments.

I have published these results in *Genes* (Strážnická *et al.*, 2018).

It is now widely accepted that phenotypic variation might not stem exclusively from variation of underlying genes *per se*, but also from genetic variation in levels of gene expression (Harrison *et al.*, 2012). In **Paper 4**, I present evidence suggesting interaction between regulatory and protein coding variation and its possible adaptive value in the bank vole. Quantitative reverse transcription PCR (RT-qPCR) was used to assess the expression levels of the two paralogous genes coding for beta-globin previously described to differ in individuals from Great Britain and to evaluate possible differences in the expression level of the paralogues depending on the individual genotype or individual locality. The significance of the existing intraspecific variability in expression of the haemoglobin coding genes for the overall intraspecific functional variability is discussed.

Paper 4 was published in *Frontiers in Ecology and Evolution* (Dvořáková, Horníková *et al.* 2020).

Paper 5 provides further support for possible existence of intraspecific adaptive variation in the bank vole and addresses the common premise of the environmental niche models - using the whole species as one ecologically undifferentiated entity - and shows why this approach should be

reconsidered to achieve more biological realism in modelling species' response to climatic variation. Using the maximum entropy based software MaxEnt, ecological niche is constructed for both the bank vole as a species and for its four major evolutionary lineages. Niche divergence between those lineages is evaluated using niche identity and similarity tests. Habitat suitability under current climatic conditions was estimated for both the species and its genetic lineages to validate the model, which was then used to estimate distribution under LGM and mid-Holocene climatic conditions. The effect of the splitting on recovering the geographic patterns of present distributions is evaluated. The distinct bank vole genetic lineages exhibit differences in their currently occupied and estimated past ecological niches and hence we discuss the importance of such intraspecific functional variability for the response of the bank vole to the end-glacial climate change and for determining the contemporary distribution patterns. The importance of the cryptic northern refugia for the bank vole survival is shown and the results in general support that treating a species as a genetically homogenous entity in ecological niche modelling might overlook important evolutionary processes below the species level.

Manuscript presenting the Paper 5 was submitted to *Journal of Biogeography* and is currently under revision (Escalante, Horníková *et al.*, under revision).

Summary of the results

Recent technological, methodological and conceptual advances in many fields of biology, such as the increasing accessibility of the genomic data and bioinformatics tools for their analysis, shift in ecological niche modelling towards the intraspecific level and emergence of functional analyses of genetic polymorphisms, enabled adopting multidisciplinary and complex integrative approaches to research on species' response to past climatic and environmental changes. In my project, I set an example of such integrative approach within the framework of adaptive phylogeography using an emerging model species, the bank vole. I searched for signs of population replacement during postglacial recolonization of Europe in the bank vole genomic DNA, investigated ecological differences between populations involved in those replacement events and also estimated possible role of a particular beneficial allele encoded in nuclear genes and selection in general in shaping the populations' reactions to the past climatic change.

Several small mammal species show patterns of genetic diversity throughout Europe that lack any general pattern indicative of a simple south-north postglacial range expansion, with the bank vole being among the species with the most complicated phylogeographic patterns (Pedreschi *et al.*, 2019). This points to a rather complex population history and several aspects of the complexity have already been described in bank voles using both mtDNA and genomic data, including survival in cryptic northern refugia (Kotlík *et al.*, 2006) and population replacement events during the recolonization of margin areas of the European distribution range (Kotlík *et al.*, 2018). Here, I for the first time used genomic data in a large scale study covering the whole species range within Europe and high number of individuals, in particular, the dataset consisted of more than 6000 genomic SNPs genotyped in more than 800 individuals from 103 localities spanning the whole European part of the bank vole distribution area. Using data with such a good geographical and genomic coverage allowed painting a more detailed picture of the bank vole colonization history than possible previously with the mtDNA markers. The results presented here demonstrate particularly the importance of the cryptic northern refugium in Carpathian Mountains for recolonization and shaping of genetic diversity of populations not only in northern latitudes (i.e. Fennoscandia, Marková, Horníková *et al.* 2020), but also in central and southern Europe (Horníková *et al.*, in prep.) and uncover previously undetected complex patterns of bank vole population history.

In Fennoscandia, the SNP data provide evidence for colonization in several waves from two glacial refugia; in particular, there were at least three independent successive waves from the Carpathian refugium via the southern land-bridge connecting Denmark and Sweden and a single colonization event via the north-eastern route through Finland from a refugium near Ural Mountains (Marková, Horníková *et al.*, 2020). Interestingly, even though the genetic diversity along the route of each colonizing population reflects serial population bottlenecks and exhibits latitudinal decrease as expected by the well-known paradigm of 'southern richness, northern purity' (Hewitt, 2000), the complex colonization history including admixture between the different colonizing populations led to high genetic variability in Fennoscandia as a whole (Marková, Horníková *et al.*, 2020). Moreover, it was not only the admixture between populations from different refugia, but also the admixture between the subsequently arriving populations from one refugium, that resulted in elevated genetic diversity in the colonized area (Marková, Horníková *et al.* 2020). These findings show the importance of not only spatial, but also temporal isolation of the species' populations and its overall colonization dynamics in preserving genetic diversity during

range expansions and also add to the mounting pile of evidence that the once popular, but also increasingly challenged paradigm of ‘southern richness, northern purity’ (Hewitt, 2000) does not apply in many species.

In central Europe, the gene flow between the bank vole populations was not interrupted by submersion of land bridges as in the case of Great Britain (Kotlík *et al.*, 2018) and southern route to Fennoscandia (Marková, Horníková *et al.* 2020), resulting in recolonization history that exhibits even more complexity than in the apex areas of the bank vole distribution range, including admixture and replacement events that are most likely an on-going process (Horníková *et al.*, in prep.). There is evidence that Carpathian refugium was an important source for colonization of the mid-latitudes, demonstrating that populations of temperate species surviving in such high-latitude refugia might have been successful in recolonization of not only the apex areas of the species distribution, but also of central Europe (Horníková *et al.*, in prep.). However the results of the Bayesian scenario choice also show that for the bank vole the Mediterranean peninsulas were not only the mere ‘hot-spots of endemism’ as thought previously based on mtDNA (Bilton *et al.*, 1998). On the genomic level, the Mediterranean populations also contributed to the present populations in central Europe by providing admixture to the genomes derived from the Carpathian refugium in several parts of Europe (Horníková *et al.*, in prep.). These findings not only stress the importance of using genomic data in species’ phylogeography and population genetic studies, as the large scale genomic data can bring important novel insights even in species with reasonably well described phylogeographic patterns derived from mtDNA, but most importantly also refine the picture of bank vole postglacial expansion through Europe and provide important implications for our understanding of the species’ response to environmental changes.

If, as the results suggest, there has been gene flow between populations originating in different glacial refugia such as the one detected between populations of Mediterranean and Carpathian origin, it may have played an important role for the species’ response to environmental changes. While the gene flow has been often deemed a rather disruptive force that can erode local adaptations by diluting the locally adapted genomes (Lenormand, 2002), an increasing number of studies show that it might as well provide benefits such as increased genetic variation, providing material for novel trait combinations or even transfer of already ‘preadapted’ alleles that might enable local adaptation and thus facilitate survival of the species under novel environmental conditions (Tigano and Friesen, 2016). In the bank vole, a very convincing example of functional variation carried by populations from different glacial refugia suggesting presence of local intraspecific adaptation is represented by the differentially expressed haemoglobin genes (Kotlík *et al.*, 2014; Strážnická *et al.*, 2018). The 52Cys allele responsible for increased resistance of red blood cells to oxidative stress segregates in both paralogous beta-globin genes (Kotlík *et al.*, 2014) and exhibits sharply delimited geographical distribution throughout the species range (Strážnická *et al.*, 2018). By mapping the distribution of the 52Cys allele and correlating it with environmental variables, my results clearly demonstrate that even though the 52Cys allele distribution in both genes is strikingly similar to the distribution of Western mtDNA, there is a significant correlation with multiple climatic variables, suggesting that it was shaped not only by the population history, but most probably also by environmental selection (Strážnická *et al.*, 2018). These results raise the intriguing possibility that the advantage of Cys haemoglobin production could be linked with levels of environmental oxidative stress (Strážnická *et al.*, 2018), where the possible trade-off between the use of Cys for haemoglobin synthesis and its use for synthesis of other biomolecules or the high reactivity of the thiol group making the Cys prone to variety of potentially harmful chemical reactions could contribute to reduced fitness in environments where the Cys allele does not provide

a strong functional advantage (Strážnická *et al.*, 2018). We propose that for the allele to be maintained in population even under less favourable conditions, the thiol level in the red blood cells is optimized by allelic and interlocus variation in Hb Cys content (Strážnická *et al.*, 2018) possibly coupled with regulatory genetic variation that would allow for fine-tuning of the phenotype in response to selection by affecting the contribution of the two beta-globin polypeptides to haemoglobin production (Dvořáková, Horníková *et al.* 2020).

While the haemoglobin coding genes remain for now the only concrete example of beneficial and presumably adaptive intraspecific genetic variation between bank vole populations originating in different glacial refugia, these results support the premise of adaptive phylogeography first postulated in 2014 based on the distribution pattern of 52Cys in Great Britain, that populations originating from different glacial refugia might have been adapted to particular conditions encountered in those refugia and that these adaptations might have affected which areas were these populations capable of recolonizing, i.e. that the process of postglacial recolonization was greatly influenced not only by Europe's geography and distance from refugia, but also by selection (Kotlík *et al.*, 2014). The ecological niche modelling performed on both the species and population level provides an independent support for the hypothesis that the refugial populations might have been locally adapted to ecological conditions encountered in the particular refugia and therefore could have played differently important role for species' ability to cope with changing environment during the end-glacial spread (Escalante, Horníková *et al.*, 2020, under revision). The intraspecific genetic lineages exhibit significant differences in their ecological niches, suggesting that the population replacement events responsible for the contemporary phylogeographic patterns in the bank vole were at least partially driven by ecological differences between the involved populations and were not purely stochastic events (Escalante, Horníková *et al.*, 2020, under revision). These results also demonstrate that assuming the characteristics of ecological niches to hold across the entire species range could potentially lead to erroneous modelling of the species' response to environment (Escalante, Horníková *et al.*, 2020, under revision) and most importantly, in combination with all the other insights presented here, they also underscore the importance of preserving as much intraspecific diversity as possible for species' survival under climate change, since with extinction of locally adapted populations, alleles that could serve as a substrate for intraspecific introgression and/or adaptation might be lost.

Conclusion and future prospects

The main aim of my thesis was to investigate possible adaptive changes in distribution of the bank vole populations in response to climate change at the end of the last glaciation. By combining large scale genomic data with information on particular intraspecific genetic variation and ecological niche modelling, I was able to bring novel insights into the bank vole population history and provide evidence supporting importance of intraspecific genetic variability between evolutionary lineages originating in different glacial refugia for species' ability to cope with climatic and environmental changes. Those insights underline the benefits of re-investigating species' histories through SNP phylogeography and focusing on intraspecific level when performing environmental niche modelling and in general demonstrate the importance of an integrative approach to the research on species' response to climate change.

The well described variation in bank vole beta-globin coding genes is presently the best studied, but certainly not the only genetic system that underlies the ecological differentiation of the different bank vole populations. The future research thus should focus on identification of other genes that would show signs of environmental selection. As a first step, the extensive SNP dataset already available (Marková, Horníková et al., 2020, Horníková et al., in prep.) could be analysed using various genome-scan methods based on environmental association for signals of selection using the significant environmental variables identified in the analysis of the environmental association of the 52Cys allele. The regions with outlier loci detected by various approaches could then be blasted on published mouse genome to identify candidate genetic regions for environmental adaptations, a cost-effective approach successfully adopted for example in fish (Dalongeville *et al.*, 2018). Since the genotyping by sequencing approach used for generating of the SNP dataset provides the polymorphic loci only from a portion of the genome and therefore important regions for adaptation could potentially be missed, the future research could also take advantage of the increasing availability of the whole genome sequencing. A reference genome already obtained by whole genome sequencing provides new possibilities for more accurate identification of the candidate genetic regions from the SNP data already available. Sequencing whole genomes of multiple individuals from populations with different ecological niches would provide truly genome-wide SNP data for investigation of signs of selection in the populations from different refugia. Anyhow, linking the candidate loci to functional genes or to coding regions of known (or presumed) functional importance could prepare the ground for thorough functional analyses similar to the analysis of the haemoglobin coding genes, i.e. for better understanding of genetic and functional basis of the ecological differentiation between the populations. Genome-wide scans for functional variation between populations representing different ecological niches could help us to resolve which genes or coding regions in particular are involved in adaptation to particular environmental conditions. As the long planned breeding facility for bank voles is becoming reality, it should soon be possible to include also the investigation of physiological consequences of the previously identified functional and putatively adaptive variation between the particular populations on the level of the whole organism, i.e. the effect on fitness. Given the possible importance of gene flow between the populations originating in different refugia as a source of standing genetic variation or even intraspecific adaptive introgression, the efforts of the future research should be directed particularly to the areas of the intraspecific contact zones, namely to Great Britain and Scandinavia.

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