Genomic signatures of climate adaptation in bank voles

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Abstract

Evidence for spatially varying selection and adaptive variation can provide insight into a species' ability to adapt to different environments. However, despite recent advances in genomics, it remains difficult to detect footprints of spatially varying selection in natural populations. Here we analysed ddRAD sequencing data (21,892 SNPs) in conjunction with geographic climate variation to search for signatures of adaptive differentiation in twelve populations of the bank vole (Myodes/Clethrionomys glareolus) distributed across Europe. To identify the loci subject to spatially varying selection and associated with climate variation, we applied multiple genotype-environment association (GEA) methods, two univariate and one multivariate, and controlled for the effect of population structure. In total, we identified 213 candidate adaptive loci, 74 of which were located within genes. In particular, we identified signatures of selection in candidate genes with functions related to lipid metabolism and the immune system. Using the results of redundancy analysis (RDA), we demonstrated that population history and climate have joint effects on the genetic variation in the pan-European metapopulation. Furthermore, by examining only candidate loci, we found that annual mean temperature is an important factor shaping adaptive genetic variation in the bank vole. By combining landscape genomic approaches, our study sheds light on genome-wide adaptive differentiation and the spatial distribution of variants underlying adaptive variation in bank voles that are influenced by local climate.

Introduction

Understanding how organisms adapt to their local environment is one of the central questions of evolutionary biology, which is becoming increasingly important in a world of human-induced rapid climate and environmental change (HIREC). It is generally accepted that genetic variation within and among populations is influenced by the local environment in which organisms reside. For example, populations along an environmental gradient may be adapted to their local conditions if selection is strong enough relative to drift and gene flow between populations (Kawecki & Ebert, 2004). Since local adaptation arises from natural selection on adaptive phenotypic traits, it can be demonstrated by genetic differentiation at the genetic loci underlying

those traits (Phifer-Rixey et al., 2018; Stillwell, 2010; Stinchcombe et al., 2004). The genetic basis for environmental adaptation has been uncovered for a few obvious traits with distinct phenotypic characteristics, such as variation in coat colour in mice in relation to environmental background colour (Linnen et al., 2009; Nachman et al., 2003) or reduction in armour plating in sticklebacks in response to freshwater colonization (Colosimo et al., 2005; Cresko et al., 2004), or body size and blood chemistry (Phifer-Rixey et al., 2018).

Theoretical and empirical studies suggest that many adaptive processes have a polygenic basis and are controlled by many genes of small effect (Barghi et al., 2020; Yeaman, 2015). Conventional genome scans are good at detecting signals from adaptive loci with large effects, but their ability to detect weak signals of polygenic selection acting across many loci is rather limited, because they focus on only one locus at a time (Rellstab et al., 2015; Wellenreuther & Hansson, 2016). In contrast, multivariate genotype-environment association (GEA) methods offer the possibility of analysing many loci simultaneously together with several environmental predictors. These are therefore suitable for detecting signals of polygenic selection (Capblancq et al., 2018; Forester et al., 2018), but have not yet been widely used in evolutionary ecology. In addition, these multivariate approaches also allow quantification of spatial patterns of adaptive genetic variation associated with environmental variables (Lasky et al., 2012; Micheletti et al., 2018; Nadeau et al., 2016).

Small forest mammals provide an ideal biological model to investigate the relative roles of selective and neutral factors in response to clinal environmental gradients, because they have large geographic ranges and individuals are not highly mobile within the range (Haasl & Payseur, 2016). Such gradients, and their respective genetic responses, include climate, and divergence in gene regulatory regions and genes related to metabolism and immunity (Phifer-Rixey et al., 2018) or body size and extremities ratio (Ballinger & Nachman, 2022); rural-urban gradients, and signals of selection in genes involved in lipids and carbohydrates metabolism (S. E. Harris & Munshi-South, 2017); as well as altitudinal gradients, and genes related to metabolic function and oxygen transport (Beckman et al., 2022; Waterhouse et al., 2018).

The bank vole *Myodes glareolus* (also known as *Clethrionomys glareolus* ; Kryštufek et al., 2020) is a small Eurasian forest-dwelling rodent with a broad geographic distribution in Europe, ranging from the Mediterranean peninsulas and the southern coast of the Black Sea in the south almost to the northern edge of Scandinavia (Figure 1). This distribution covers a wide temperature gradient (Figure 1). Bank voles survived in several refugia during the Last Glacial Maximum, including the well known refugia on the Mediterranean peninsulas and cryptic refugia in the Carpathians (Kotlik et al., 2006; Wójcik et al., 2010) and the Ural Mountains (Abramson et al., 2009; Deffontaine et al., 2005). Their subsequent recolonization of Europe, when the climate became more favourable at the beginning of the Holocene, resulted in a complex genetic structure, with several distinct phylogeographic lineages, first described based on mitochondrial DNA sequences (Filipi et al., 2015) and later confirmed by genome-wide SNP analyses (Hornikova et al., 2021; Markova et al., 2020). Bank voles have limited dispersal capabilities (Deter et al., 2008; Viitala et al., 1994) and short generation times, resulting in large local effective population sizes. Together with their limited capacity to disperse, these factors result in a large evolutionary potential for genetic responses to local conditions. Therefore, bank voles are a suitable system to study signatures of local adaptation in response to spatially varying climate-induced selective pressures along an environmental gradient. They have been the target of GEA-studies in relation to geographic expansion (White et al., 2013) and tolerance to *Puumala orthohantavirus* infection (Rohfritsch et al., 2018). However, the specific selection forces driving adaptations, as well as the genetic loci involved, are not well understood for wide latitudinal gradients.

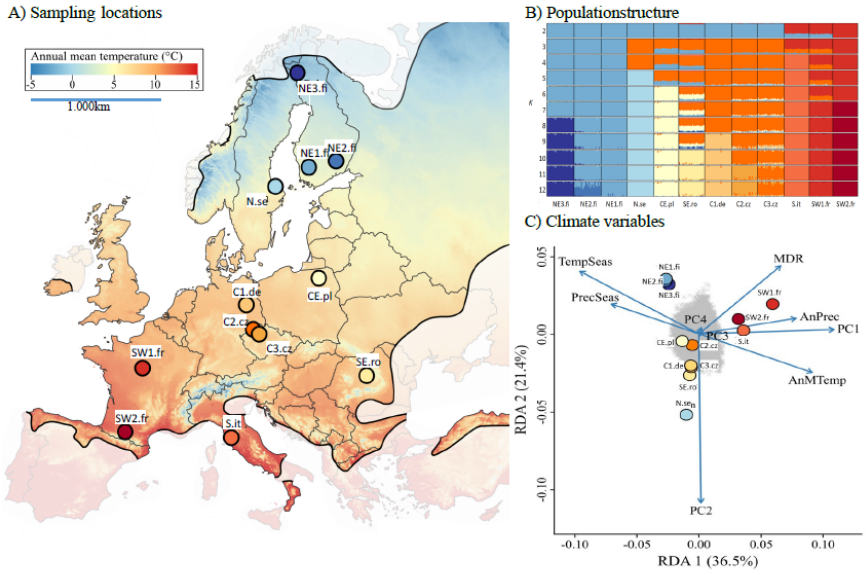


Figure 1. A) Figure 1. Sampling locations of populations (coloured circles) of*Myodes glareolus* in Europe with annual mean temperature (data: www.worldclim.org (Fick & Hijmans, 2017) and current distribution (Shenbrot & Krasnov, 2005). B) Admixture proportions using NgsAdmix based on 2,476 SNPs, with various numbers of ancestral populations (K= 2-12). Each individual is represented by a column with colours corresponding to the proportions of their ancestry components. Vertical black bars separate putative populations based on sampling location C) Ordination plots from Multivariate Redundancy Analysis (RDA) of the first two constrained axes summarizing with 21,892 SNPs (in grey), including five climate variables (Annual mean temperature AnMTemp, mean diurnal temperature range MDR, Temperature seasonality TempSeas, Annual precipitation AnPrec, precipitation seasonality PrecSeas) and four axes of population structure. Abbreviations - C: Central, E: Eastern, N: North, S: South, W: Western , .fi: Finland, .se: Sweden, .pl: Poland, .cz: Czechia, .de: Germany, it: Italy, .fr: France, .ro: Romania

The aim of this study is to investigate how population history and adaptation to local climate affect the spatial distribution of genomic variation in bank vole populations across Europe. We identified candidate genes and climate variables responsible for adaptive variation by testing for associations between allele frequencies and environmental variables, using multiple univariate and multivariate GEA methods. We estimated the relative role of population structure versus environmental selection in explaining observed genetic variance by accounting for the neutral genetic structure.

Methods

Vole samples and climate variables

Tissue samples of a total of 275 voles representing 12 widely dispersed populations (Figure 1) with 21-24 individuals per population (Table S1) were collected by the authors, covering a distance of 3,200 km from the northeast in N Finland (25.9°E 68.0°N) at the northern distribution limit to the southwest in France (0.8°E 43.2°N) and 2,700 km from the southwest (France) to the southeast in Romania (25.1°E 46.6°N). For each population, values of 10 bioclimatic variables were downloaded from the WorldClim V2 dataset (Fick & Hijmans, 2017). Climate at the sampling sites ranged for mean annual temperature from -2°C to 12.5°C, for temperature seasonality from 500 to 1000 (SD*100), for mean diurnal temperature range from 5.5°C to 9.8°C, for mean annual precipitation from 480mm to 1080mm, and for precipitation seasonality from 11% to 50% (Figure 1). To account for correlation among climate variables (Table S9), principal component analysis (PCA) was used to reduce dimensionality in R v3.4.4 using the prcomp function (R Core Team, 2018) [2]

(Figure S1). This resulted in two climate-based principal components that together explained 80% of the total variation. PC1 explained 62.5% of the variation and was mainly associated with temperature variables, while PC2 explained 17.1% of the variation and was mainly associated with precipitation variables (Table S2).

Molecular methods

DNA was extracted using the DNEasy Blood & Tissue kit (Qiagen, Hilden, Germany), quantified using a Qubit 2.0 fluorometer (Life Technologies Inc., ON, Canada), and subjected to a double digest restriction associated DNA (ddRAD) sequencing protocol (Peterson *et al.* , 2012, Supplement M1). Samples were grouped into pools of 48 individuals and cleaned with Speedbeads. Each pool was size-selected for fragments of 300-400 bp in length using a Pippin Prep system (Sage Science, Beverly, MA, USA). This range should yield approximately 38,000 ddRAD loci, based on *in silico* digestion of the *C. glareolus*genome sequence (GCA_-001305785.1) using SimRAD (Lepais & Weir, 2014). For each pool, we performed qPCR to determine the optimal number of PCR cycles based on the onset of the saturation phase on amplification plots (range: 11-14 cycles) (Gansauge & Meyer, 2013). Pools were then amplified in four parallel reactions of 40 µl with primers that amplify only fragments containing both P1 and P2 adapters. The resulting libraries were sequenced in two separate runs on an Illumina NextSeq 500 with mid-output kits. We first sequenced the libraries with 75 bp paired-end (PE) and then performed another sequencing run with 150 bp PE sequencing.

Mapping and SNP calling

Cutadapt v1.4 (Martin, 2011) was used to remove adapter sequences and trim bases with a Phred score of less than 20 at the 3' end, retaining only reads with a minimum length of 35 bp. These were then demultiplexed and assigned to individuals using iPyrad v0.7.13 (Eaton, 2014). To increase mapping success, we used the published *M. glareolus*reference genome, which we improved with the pipeline Cross-Species Scaffolding (Grau et al., 2018) using the prairie vole (*Microtus ochrogaster*) reference genome (GCA_000317375.1, Table S3), Only biallelic SNPs with a minimum base quality of 20, minor allele frequency greater than 0.05, minimum p-value threshold for calling a SNP of 10^{-6}, minimum read depth of 5, and maximum read depth of 100 per sample were considered. In addition, a site had to be present in at least 12 individuals in each of the 12 populations to be considered.

Genetic diversity and population structure

We estimated diversity statistics within populations using ANGSD (Korneliussen et al., 2013). First, we calculated nucleotide diversity as the average number of pairwise differences (π) (Nei & Li, 1979) and as the proportion of segregating sites (ϑ_W) (Watterson, 1975). We calculated heterozygosity for each individual based on its site frequency spectrum (SFS) and estimated the inbreeding coefficients (F) using ngsF (Vieira et al., 2013).

To assess population structure using PCA, we created a covariance matrix among individuals using ngsCovar from the ngsTools suite (Fumagalli et al., 2014) and calculated principal components in R v3.4.4 (R Core Team, 2018) using the 'eigen' function. The number of principal components explaining most of the population structure was determined from the scree plot of PCA (Cattell, 1966).

We assessed admixture among populations using NGSadmix (Skotte et al., 2013) with a number of clusters K ranging from 2 to 14. We repeated each analysis 20 times and reported the results of the highest likelihood analysis for each K . Finally, we calculated the pairwise F_{ST} in ANGSD using the shared site frequency spectrum for each pair of populations. The results were also used to test for isolation by distance (IBD) by calculating the correlation between pairwise linearized F_{ST} values [$F_{ST}/(1-F_{ST})$] and log-transformed pairwise geographic distance (Rousset, 1997) using a Mantel test with 1,000 permutations in the R package '*Vegan 2.5-4* ' (Dixon, 2003; Table S4)

Identification of loci associated with climate variation

To identify loci subject to climate-induced selection, we searched for genomic markers that showed the

strongest association between allele frequencies within populations and climatic condition in the respective population. We used the univariate approaches Bayenv2 (Coop et al., 2010) and LFMM (Frichot et al., 2013) together with multivariate redundancy analysis (RDA). RDA allows the analysis of multiple environmental variables and covarying selection signals across a set of multiple loci and facilitates the detection of signatures of polygenic selection (Forester et al., 2018) and was performed using the package 'Vegan 2.5-4' in R (Dixon, 2003). As a conservative approach, we only considered candidate adaptive loci that were detected by at least two methods (Supplement M1, de Villemereuil et al., 2014).

Partitioning genetic variation between population structure and climate

The percentage of variation explained by the joint effect of climate and population structure was calculated by subtracting their independent effects from the total variation explained. We performed a partial redundancy analysis (pRDA) to separate the effect of climate from that of population structure, calculating the genetic variation explained by each climate variable by conditioning its effect on the other variables. The significance of the models was tested using ANOVA with 1,000 permutations. To identify the climate variables that contributed most to adaptive variation, we performed pRDA on the set of loci identified as outliers. Finally, to identify the climate variable that was most strongly associated with variation at each outlier locus, we extracted loci scores from the separate pRDA for each climate variable and normalized these scores to a mean of zero and a unit variance. We then considered the climate variable with the highest absolute value to be the one with the strongest influence on that locus.

Cumulative adaptive variation

We calculated polygenic scores for each individual to assess the cumulative contribution of each locus to the response to climatic conditions using the custom script in R [PK1] (Babin et al., 2017; Gagnaire & Gaggiotti, 2016). For each candidate adaptive locus, we first identified the favourable allele as the allele with a frequency that was positively associated with increasing values for a given climate variable (Hancock et al., 2011). Polygenic scores were calculated at the individual level by summing the total number of favoured alleles for a given climate variable, resulting in an independent score for each climate variable. To ensure loci independence, we used only one locus per RAD tag, but also performed the same analysis with loci obtained by all methods for comparison. To examine the effects of population structure on RDA results, we separately calculated polygenic scores for the outlier loci detected by RDA with and without correction for population structure (Table S7).

SNP annotation and gene ontology

To obtain functional annotations for candidate loci, we used the LastZ pairwise alignment tool v1.04.00 (R. S. Harris, 2007) to find homologous *M. ochrogaster* positions. To do this, we used a 20,000 bp scaffold surrounding each candidate locus as a query in LastZ and the default options for calculating pairwise alignments. Only alignments with a bit score greater than 1,000 and a query coverage of at least 50% were considered. If multiple alignments passed this filter, the alignment with the longest length and highest bit score was selected as the best match. If the loci were on protein-coding regions, we used the UniProt database to examine gene function and find gene ontology (GO) terms. We performed an enrichment analysis using topGO (Alexa & Rahnenführer, 2010) in the "biological processes" category. We compared our list of candidate genes with all genes with a SNP. We used Fisher's exact test and the *elim* algorithm to account for correlation in the topology of the GO graph, and reported the GO terms with a p-value < 0.01 and at least 4 associated genes (Table S6).

Results

SNP dataset

We obtained 592.4 million reads from the two runs of sequencing. After filtering for low quality reads and assigning individuals to barcodes, on average 1,62 Mio reads per individual aligned to our improved reference genome, resulting in between 0.96 Mio (Site NE1) and 2.61 Mio (C1) reads per population. Across all individuals, high quality reads covered an average of 17.99 Mio nucleotides of the genome (˜0.71%).

Average number of sites for each individual ranged from 3.1 Mio to 15.5 Mio, with an average of 8.6 Mio per individual. Using this data, a total of 21,892 SNPs present on 7,679 RAD-tags passed filtering and were used in the genotype-environment analyses. The dataset used to examine population structure consisted of 2,476 variable sites with data for all individuals.

Genetic diversity within populations

The proportion of segregating sites (ϑ_W) ranged between 161). The average number of pairwise differences (π) ranged between 0.0019 (N.fi) [3] and 0.0041 (S.it), with an average of 0.0030. Observed heterozygosity across populations ranged from 0.0012 (N.fi) to 0.0025 (SE.ro). None of the genetic diversity measurements showed any clear spatial pattern. Inbreeding coefficients (F_{IS}) were overall low.

Table 1. Overview of nucleotide diversity estimates (mean ± standard deviation) for each of the 12 sampled *Clethrionomys glareolus*populations. Sample size (n), Watterson's theta (ϑW), heterozygosity - the proportion of heterozygous genotypes and the average population inbreeding coefficients (F_{IS}) are displayed.

	Site	Country	n	θW (*10^{-3})	Heterozygo	FIS
	NE3	Finland (.fi)	24	2.6 (1.6)	1.9 (0.07)	0.014 (0.030)
	NE2	Finland (.fi)	24	2.5 (1.5)	1.8 (0.06)	0.004 (0.011)
	NE1	Finland (.fi)	23	2.4 (1.5)	1.6 (0.13)	0.007 (0.014)
	N	Sweden (.se)	24	1.6 (1.2)	1.2 (0.10)	0.003 (0.007)
	CE	Poland (.pl)	22	2.8 (1.5)	2.1 (0.03)	0.016 (0.025)
	SE	Romania (.ro)	23	4.8 (2.1)	2.5 (0.16)	0.028 (0.023)
	C1	Germany (.de)	24	2.6 (1.4)	2.0 (0.07)	0.025 (0.034)
	C2	Czechia (.cz)	24	3.5 (1.9)	2.1 (0.15)	0.012 (0.026)
	C3	Czechia (cz)	23	3.3 (1.7)	2.1 (0.08)	0.018 (0.025)
	S	Italy (.it)	22	3.5 (1.8)	2.5 (0.16)	0.012 (0.029)
	SW1	France (.fr)	21	2.9 (1.4)	2.1 (0.11)	0.006 (0.012)
	SW2	France (.fr)	22	2.0 (1.3)	1.6 (0.13)	0.003 (0.011)

Population structure

PCA of 2,476 loci based on genotype likelihood clustered individuals broadly based on geography along the first 4 principal components (Figure 2), which together explained 20.1% of the genetic variation.

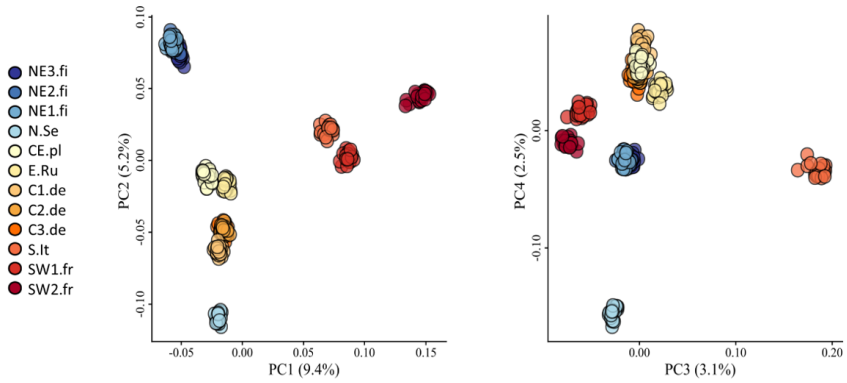


Figure 2. Principal component analysis of 276 *Clethrionomys glareolus* individuals sampled from 12 populations across Europe using 2,476 SNPs based on genotype likelihood. With percentage of variation explained

for each component displayed on the axes, together the four components explain 20.1% of variation. Each circle represents an individual, colours correspond to Regions and sites Abbreviations - C: Central, E: Eastern, N: North, S: South, W: Western, .fi: Finland, .se: Sweden, .pl: Poland, .cz: Czechia, .de: Germany, it: Italy, .fr: France, .ro: Romania

PC1 explained 9% of the variation and sorted the populations by geography, correlating to both longitude (R^2= 0.83) and latitude (R^2=0.49). PC2 (5%) separated West and East within Regions; in the Northern populations the population west of the baltic sea (N.se) from the the three Northern populations east of the Baltic sea (NE1-3.fi), and within Central Europe the three geographically close populations (C1.d3, C2-3.cz) from the two Eastern European populations (CE.pl and SE.ro). The third and fourth components together explained 5.5% of genetic variation, and separated two populations from the other ten: the northern (N.se) population beyond the Baltic Sea, and the southern population beyond the Alps (S.it).

In the admixture analysis (Figure 1), the lowest variance of likelihood was found for 2 or 3 ancestral populations (Figure S2). Assuming$K$ =2, the populations in the southern range of the distribution are separated from the other populations. Increasing to K =3 additionally separated the three populations east of the Baltic (NE1-3.fi), increasing to K=4 separated S.it beyond the Alps, and to K=5 the Swedish population beyond the baltic (N.se). Assuming$K$ =10 (having the next lowest variance of likelihood), clusters mirrored sampling locations, except for the two pairs of populations with the least geographic distance, the southern Finnish populations forming a single cluster, and the Central populations (C2 and C3.cz) with only some degree of admixture suggested.

A similar pattern emerges from the pairwise population F_{ST} estimates (PPF), which revealed moderate to high levels of differentiation between populations (Table S4). PPF ranged between 0.035 (C1.cz vs C2.cz) and 0.555 (N.se and SW1.fr) with an average fixation index of 0.269 (SD = 0.12). PPF corresponded well with geographic proximity of populations (Mantel tests genetic and geographic distance correlation: r = 0.47, p = 0.002), suggesting a strong spatial pattern of isolation by distance. In accordance with previous results, the population from Sweden (east of Baltic sea) was more similar to the central European populations than to the North-Eastern populations.

Candidate loci associated with climate

The univariate approaches identified a total of 975 loci associated with climate variables. Bayenv2 identified a total of 631 outlier loci; of these, 283 loci were associated with climate-based PC1 and 354 loci were associated with climate-based PC2. To run LFMM, we first determined the appropriate number of latent factors using snmf. The snmf analysis returned the lowest CE value for K =10 (0.519), followed by$K$ =11 (0.520) and K =12 (0.521) (Figure S3). As higher values of K resulted in a higher number of outlier loci, we only report outlier loci detected using K =10 as a conservative approach. LFMM identified a total of 497 outlier loci, among which 134 loci were associated with climate-based PC1 and 377 with climate-based PC2. Of the outlier loci identified by LFMM, 152 were also identified by Bayenv2, corresponding to 15.6% overlap between the two methods. The multivariate pRDA identified 485 outlier loci associated with the first 2 RDA axes. Among these, 69 loci were also identified by univariate approaches. Thus, 5.0% of loci were identified by both approaches. The RDA without correcting for population structure identified 108 loci as outliers, with only 8 loci identified by univariate approaches as well (0.8% overlap with univariate methods) and an overlap of only four loci with the RDA with correction for population structure. Overall, a total of 1,392 outlier loci corresponding to 1,003 RAD-tags were associated with climate using all methods (Venn Diagrams Figure S1). We considered 213 loci detected by at least two methods as strong candidates.

Annotation of candidate loci and gene ontology

Of the 213 candidate loci, 209 were successfully aligned to the *M. ochrogaster* genome. Eight loci were located in exons, 86 in introns, and 115 in intergenic regions. We identified 74 genes associated with the candidate loci, some genes contained several loci (Table S5). Several of these genes were associated with functions in lipid metabolism, energy homeostasis, and immunity (Table 2). Among GO terms associated with the genes, six were significantly enriched in our data set, including "regulation of respiratory burst"

and "dicarboxylic acid transport" (Table S6).

Table 2. Candidate genes detected by at least two genome scan methods, and associated with lipid metabolism, energy homeostasis and immunity. Locus refers to the location on the *M. ochrogaster* reference genome (Chromosome_base-position). Method of outlier detection (L: LFMM, Ba: Bayenv2, RDA: Redundancy Analysis)

Locus and Method	Gene, putative Function and relevance	Reference
NC_022011.1_19197181	Zinc finger homeobox 3 (Zfhx3)	Balzani 2016
L1, L2, Ba1	Transcription factor expressed in the suprachiasmatic nuclues with a role in circadian rhythms	
NC_022013.1_77189997	Basic leucine zipper ATF-like transcription factor 3 (Batf3)	Murphy 2013
L1, Ba1, Ba2, RDA	Transcription factor involved in the differentiation of T helper cells	
NC_022013.1_78476899	Potassium voltage-gated channel subfamily H member 1 (Kcnh1)	Zhang 2014
L2, Ba2	Involved in adipogenic differentiation and production	
NC_022017.1_17053779	ADAM metallopeptidase with thrombospondin type 1 motif 20 (Adamts20)	Silver 2008
L2, Ba2	Required for melanoblast survival, responsible for coat colour variation	
NC_022018.1_59867563	Neurotrophic receptor tyrosine kinase 2 (Ntrk2)	Xu 2016,
L2, Ba2, RDA	Critical for maintaining energy homeostasis by controlling food intake and body weight	Houtz 2021
NC_022024.1_33246556	Insulin like growth factor 1 (Igf1)	Baker 1993,
L2, Ba2	Involved in energy metabolism and mediating growth and development	Laron 2001
NC_022027.1_54997482	Leucine rich repeat containing 8 VRAC subunit C (Lrrc8c)	Tominaga 2004
L2, RDA	Associated with early stage adipocyte differentiation	
NC_022028.1_49631929	Dynein axonemal heavy chain 8 (Dnah8)	Sohle 2012
L2, Ba2	Axonemal dynein influencing lipid metabolism, possibly by reg. of inflammatory processes	
NC_022031.1_30606927	BTB domain and CNC homolog 2 (Bach2)	Kuwahara 2016,
L1, L2, Ba2, RDA	Transcription factor that acts as a broad regulator of the immune homeostasis	Yamashita 2018
9	Biliverdin reductase A (Blvra)	Baranano 2002
L2, Ba2	Facilitates conversion of biliverdin to bilirubin protecting against cell damage	
NW_004949099.1_1813079	Aprataxin and PNKP like factor (Aplf)	Grundy 2013
L1, Ba1	Involved in double-strand DNA break repair by promoting non-homologous end joining	
NW_004949106.1_3445183	Phospholipase C like 1 (Prip)	Oue 2016,
Ba1, RDA	Modulates fat metabolism and regulates non-shivering thermogenesis in brown adipose tissue	
NW_004949106.1_7957839	Signal transducer and activator of transcription 4 (Stat4)	Kaplan 2005
L2, Ba2	Encodes a transcription factor responsible for T-helper cell development	Kanematsu 2019
NC_022012.1_47630215	Exophilin 5 (Exph5)	McGrath 2012,
LPC1, BPC1, RDA	Plays a role in intracellular vesicle trafficking, mutations in this gene lead to skin fragility	Yudin 2017
NC_022030.1_4789630	Solute carrier family 2 member 12 (Slc2a12)	Gil-Iturbe 2019,
BPC1, RDA	Contributes to insulin-stimulated glucose uptake in skeletal muscle and adipose tissue	Stepanov 2017
NC_022013.1_70231999	Dual specificity phosphatase 10 (Dusp10)	Zhang 2004
BPC1, RDA	Regulator of both innate and adaptive immune resp., req. for T-cell activation and proliferation	
NW_004949164.1_394094	Engulfment and cell motility 1 (Elmo1)	Sarkar 2017
BPC1, RDA	Defence mechanisms against invading pathogens by inducing inflammatory responses	

Variance partitioning and identification of important climate variables

The RDA including all loci with climate and population structure as predictors explained 71.4% (adjusted $R^2 = 0.714$) of the genetic variance among populations ($F = 4.06$, $p > 0.001$) (Figure 1c). Using partial redundancy analysis on the full data set, we found an effect of population structure after controlling for the effects of climate ($F = 3.02$, $p = 0.01$), explaining 38.2% of genetic variance. However, we found no effect of climate alone after controlling for the effects of population structure ($F = 1.34$, $p > 0.2$). Each climate variable independently explained only a small amount of the total genetic variance (Table 3).

In the subset of 485 outlier loci that were detected by the global RDA analysis, the full RDA model explained 51.1% of the genetic variance in these outliers ($F = 3.73$, $P = 0.03$). Climate accounted for 46.7% of the total amount of genetic variation, and the joint effects of climate and population structure was reduced to 9.8% (Figure S2).

Table 3. Amount of genetic variance explained by each climate variable after removing the effect of the other variables including population structure. Results are based on a partial redundancy analysis (pRDA) of

climate variables after controlling for confounding population structure for all 21,892 SNPs, or 485 outlier SNPs identified by the RDA analysis.

Variable	All 21,892 SNPs			485 RDA outlier SNPs		
	r2	adjusted r2	P-value	r2	adjusted r2	P-value
Annual mean temperature (AnMTemp)	0.04	0.06	0.28	0.17	0.52	0.034
Temperature Seasonality (TempSeas)	0.03	0.01	0.43	0.04	0.07	0.228
Mean diurnal temperature range (MDR)	0.04	0.06	0.27	0.12	0.35	0.058
Annual precipitation (AnPrec)	0.04	0.05	0.29	0.08	0.20	0.128
Precipitation Seasonality (PrecSeas)	0.04	0.04	0.29	0.10	0.29	0.067

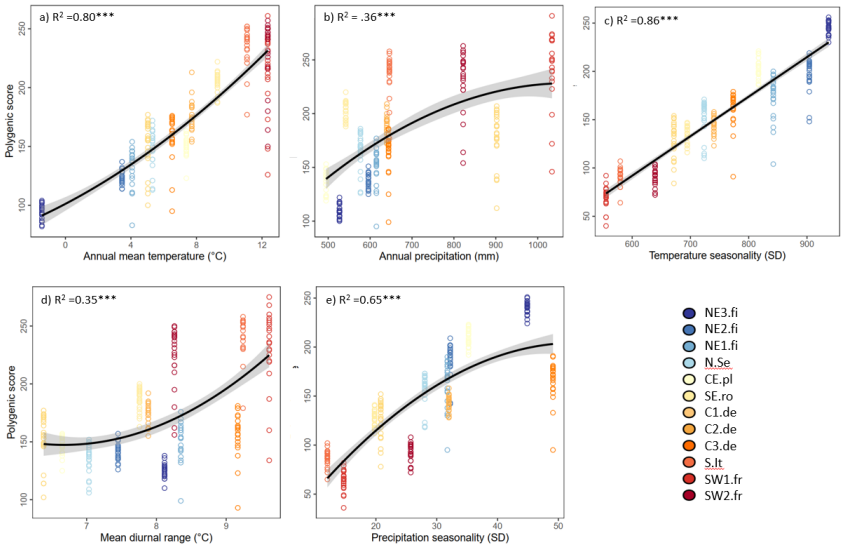
Among the climate variables, annual mean temperature explained the largest (52.0%) and only significant ($F = 6.95$, $p < 0.05$) amount of genetic variance in outlier loci. This was followed by trends for an effects of mean diurnal range ($F = 5.01$, $p = 0.06$) and precipitation seasonality ($F = 4.33$, $p = 0.07$), which respectively explained 35.1% and 29.1% of genetic variance (Table 3). The climate variable with the strongest influence on outlier loci as detected by RDA was annual mean temperature, which was associated with 37.1% of the markers, followed by mean diurnal range which was associated with 20.8% of markers.

Among the outlier loci detected by all methods, the primary associations were with annual mean temperature (27.5%) and annual precipitation (25.4%). The same picture arises if only candidate loci that were detected by at least two methods are considered. Here, 28.6% of loci are associated with annual mean temperature and 25.4% are associated with annual precipitation (Table S7).

Polygenic scores

We investigated the predictive value of each climate variable for the respective polygenic score based on 213 candidate loci, adjusting linear or quadratic models, selecting the model with the lowest Akaike information criterion (AIC) value. regressions were highly significant, and adjusted R^2 values ranged between 0.35 and 0.86 (Figure 3). Analysis using all the 1,392 detected outliers resulted in similar positive regressions, independent of whether or not we corrected for the population structure (Table S8).

Figure 3. Correlations between individual additive polygenic scores (symbols) based on 213 candidate loci and each of the five explanatory climate variables determined per sampling site: Annual mean temperature (a), Annual precipitation (b), Temperature seasonality (c), Mean diurnal temperature range (d), and Precipitation seasonality (e). Polygenic scores were obtained by summing total numbers of favorable alleles. The line represents the regression line from the model, while the shaded area represents the 95% confidence interval. Variance explained and p-values for the linear (c) or quadratic regression (a, b, d, e) fits are given in the respective upper-left corner.



Discussion

The aim of the present study was to investigate genome-wide patterns of adaptive variation associated with climate in european bank vole populations. Searching for potentially adaptive loci in a multivariate framework is a powerful approach, especially because many adaptive traits are likely to be polygenic in nature (Barghi et al., 2020; Wellenreuther & Hansson, 2016). We used ddRAD sequencing and a combination of landscape genomic approaches to uncover environment-related evolutionary processes in the bank vole. We characterized adaptive genetic variation by using univariate GEA methods to detect outlier loci correlated with climate. We identified 74 genes of interest and functional annotation suggested that energy homeostasis and response to pathogen infection are important targets of spatially varying selection in the bank vole. In addition, we have shown that both population structure and climate play important (and common) roles in explaining neutral genetic differentiation across the bank vole range. Genetic variation among candidate loci was mainly associated with variation in annual mean temperature, highlighting the importance of this climatic variable in bank vole adaptation.

Loci with true selection signals must be distinguished from loci that exhibit genetic differentiation between populations caused by neutral forces. Correcting for these effects is an important concern when identifying candidate loci subject to selection. Proper correction can help to avoid possible spurious detection of candidate loci whose allele frequencies resemble signals of selection but are the result of neutral processes due to the shared history of populations (de Villemereuil et al., 2014). We therefore used GEA methods that correct for such confounding effects by accounting for population genetic structure in the bank vole.

Agreement between the different univariate methods was reasonable (14.9% and 15.1% of SNPs were detected by both methods for PC1 and PC2, respectively) and was consistent with results observed in other empirical studies using univariate methods (Harrison et al., 2017; Prates et al., 2018; Pritchard et al., 2018). The overlap between univariate methods and RDA was relatively small, with only 5.1% of the loci detected by RDA being also detected by LFMM or Bayenv2. A simulation-based study that tested the performance of univariate and multivariate GEA methods showed that the performance of these approaches varied depending on the strength of selection (Forester et al., 2018) and that RDA may be more robust to our sampling design that does not maximize environmental differentiation. The same study also suggests that combining results from univariate and multivariate approaches may help to increase power and to reduce false-positive rates. Our study supports these findings and also provides a strong argument for using multiple approaches when searching for signals of local adaptation in highly structured populations.

Population structure explained a large part of genomic variation, resulting in a strong pattern of isolation by distance. Even after taking into account the effects of climate variation, population structure still accounted for 33% of the total genomic variation explained by the RDA. These results are not surprising, as bank vole populations experience recurring population crashes and effective gene flow between populations is generally low, which results in isolation by distance across both smaller and larger geographic scales (Aars et al., 1998; Gerlach & Musolf, 2000; Guivier et al., 2011; Redeker et al., 2006), and although populations have strongly diverged in space since the last glaciation (for review Kotlik et al., 2022)). On the other hand, climate explained 18.3% of total genomic variation when taking population structure into account, indicating association between bank vole genetic variation and environmental gradients. A large proportion of genetic variation (48.7%) could not be attributed either to the effects of climate or to spatial population structure alone, indicating that a large proportion of genomic variation associated with climate is geographically structured. The phylogeography of *C. glareolus* is marked by distinct genetic lineages, which resulted from survival within glacial refugia and recolonization of Europe at the end of the last glaciation (Filipi et al., 2015; Horniková et al., 2021; Kotlik et al., 2006; Marková et al., 2020). Post-glacial expansion from glacial refugia may result in clines of neutral allele frequencies coinciding with climate variables related to geography (Lotterhos & Whitlock, 2014; Rellstab et al., 2015), possibly explaining the large proportion of genomic variation. Our results suggest that annual mean temperature is an important driver of adaptive genomic variation and thus may be an important selection pressure influencing adaptation in the bank vole populations (Tiffin & Ross-Ibarra, 2014) as reflected by the strong association between temperature and polygenic scores. The latter implies that different alleles are maintained in different thermal environments, suggesting the presence of spatially varying selection pressure.

Temperature is one of the most important environmental factors affecting physiological processes such as the aerobic scope (Portner, 2001) and metabolism (Lovegrove, 2003), which in turn affect a variety of life history traits (Simons et al., 2011; Tokolyi et al., 2014). Numerous studies have associated clinal temperature variation and genome scans and found signals of selection in genes related to energy homeostasis and metabolism in endotherms (e.g., Andrew et al., 2018; Fumagalli et al., 2015; Hancock et al., 2011; S. E. Harris & Munshi-South, 2017; Harrison et al., 2017; Lv et al., 2014). This suggests that temperature is one of the most important environmental variables driving local adaptation. Indeed, temperature has been linked to adaptive genetic variation in other small mammals, such as populations of the recently introduced house mouse (*M. musculus*) along a latitudinal cline in eastern North America (Phifer-Rixey et al., 2018) and populations of the climate-sensitive American pika (*Ochotona princeps*) along an altitudinal cline (Waterhouse et al., 2018). The distribution and abundance of *C. glareolus* from the Eastern lineage in a contact zone with the Carpathian lineages correlated negatively with July temperature, suggesting that these individuals are better adapted to cooler conditions (Tarnowska et al., 2016), supporting temperature as a driver of adaptive genetic variation in the bank vole.

Artificial selection experiments for higher aerobic exercise performance in bank voles resulted in an increase in resting metabolic rate and thus resulted in the development of increased cold tolerance (Sadowska et al., 2015; Stawski et al., 2017). This argues for a genetic basis for thermal adaptation in bank voles that may allow individuals under natural conditions to adapt to colder environment by having more energy available for thermogenesis (Stawski et al., 2017). Although the selection regime increased cold tolerance, it also decreased the ability to thermoregulate at higher temperatures (Grosiak et al., 2020). This suggests that warmer temperatures may also be difficult for small mammals to cope with, as this can easily lead to overheating (Rezende et al., 2004). This in turn could also lead to specific metabolic adaptations in populations at warmer climates due to increased selection pressure. In this study, AMT differed between -1.4degC (NE3.fi) and 12.4degC (S.it) among populations. Thus, bank vole populations in Europe are exposed to different environmental temperatures, likely resulting in different energetic requirements and adaptive genetic divergence in metabolic traits throughout the species' range.

We have identified a number of promising candidate genes that could be considered for future research aimed at linking phenotypic and genotypic variation. The function of these candidate genes provides insight into the physiological processes that may have experienced selection across climatic gradients. Different populations

are exposed to different local climatic conditions that determine the energy requirements, diet and different pathogen or predator communities. In this context, we have identified a number of candidate genes related to lipid metabolism and the immune system that appear to be subject to temperature-related selection.

Adipose tissue plays an important role in energy homeostasis and accounts for a large portion of the energy reserves of small mammals (Birsoy et al., 2013; Sethi & Vidal-Puig, 2007). In particular, brown adipose tissue is important for metabolic heat production through non-shivering thermogenesis under cold conditions (Cannon & Nedergaard, 2004; Klaus et al., 1988). Two candidate genes associated with lipid metabolism are therefore of particular interest : the *PRIP* gene encodes an enzyme that modulates lipid metabolism and serves as a signalling molecule for non-shivering thermogenesis (Kanematsu et al., 2019; Oue et al., 2016), and *LRRC8C* which encodes a structural component of the volume-regulated anion channel in adipocytes and is associated with the early phase of adipocyte differentiation and diet-induced obesity (Hayashi et al., 2011; Tominaga et al., 2004).

Other candidate genes with functions related to energy homeostasis include *NTRK2* , which encodes the TrkB-receptor critical for maintaining energy homeostasis by controlling food intake and body weight and is responsible for regulating adaptive thermogenesis (Houtz et al., 2021; Xu & Xie, 2016). Finally, the product of *IGF1* has wide ranging effects on metabolism by coordinating protein, carbohydrate, and lipid metabolism in a variety of different cell types (Baker et al., 1993; Laron, 2001). Several of the candidate genes are associated with obesity in humans including *DNAH8* (Sohle et al., 2012), *IGF1* (Berryman et al., 2013), *KCNH1* (Vasconcelos et al., 2016), *LRRC8C* (Hayashi et al., 2011), *NTRK2* (Gray et al., 2007) and *PRIP* (Yamawaki et al., 2017), suggesting that they play a role in controlling energy homeostasis.

The results showed significant enrichment of genes related to the regulation of respiratory burst. The respiratory burst plays an important role in the immune system. It is a crucial reaction that occurs in phagocytes to degrade internalised pathogens after phagocytosis (Iles & Forman, 2002). In this context, we have identified a number of candidate genes that play important roles in the immune system. For example, the product of *DUSP10* , which was associated with this significant GO term, plays an important role in regulating both innate and adaptive immune responses through its regulatory influence on the MAPK pathway (Arthur & Ley, 2013; Seternes et al., 2019). Two other candidate genes, *BATF3* and *BACH2* , both encode transcription factors that regulate T helper cell function. Interestingly, they also interact with each other to bind to regulatory regions of cytokine gene loci and prevent excessive T helper response (Kuwahara et al., 2016; Yamashita & Kuwahara, 2018). Another candidate gene of interest is *STAT4* . This gene encodes a transcription factor responsible for the differentiation of T helper cells (Kaplan, 2005)and is part of the JAK-STAT signalling pathway that controls the immune response to viral infections (Villarino et al., 2017). JAK-STAT is one of the significantly enriched signaling pathways associated with Puumala hantavirus infection in the bank vole (Rohfritsch et al., 2018). It has also been found to play a role in the immune response to Sin Nombre hantavirus in deer mice (*Peromyscus maniculatus*) (Schountz et al., 2012, 2014). This suggests that alterations in this gene may be related to Puumala hantavirus infections in the bank vole populations we studied. Similar evidence for differential selection on immune-related genes has been observed in bank vole populations along environmental gradients at both broad and local scales, using candidate genes (Dubois et al., 2017; Guivier et al., 2014) and more exploratory genome-wide approaches (Rohfritsch et al., 2018; White et al., 2013). For humans, the diversity of the local pathogenic environment is the predominant driver of local adaptation (Fumagalli et al., 2011) and we may speculate that pathogen environment and pathogen pressure is closely associated to climate variation, with rapid adaptations expected due to climate change. Taken together, the selection signals in the most promising candidate genes (Table 2, full overview in Table S5) suggest that the energy balance and immune system in the bank vole are important targets of temperature-mediated, spatially varying selection.

Conclusions

In this study on genomic adaptation of a small mammal to a pan-European climate gradient, we have shown that both geographic population structure and climate play important roles in explaining neutral genetic differentiation across the bank vole range. Genetic variation among candidate loci was mainly explained by

variation in annual mean temperature, highlighting its importance for climate adaptation in the bank vole. We identified 74 genes that showed evidence of spatially varying selection and whose functional annotation suggested that energy homeostasis and response to pathogen infection are important targets of spatially varying selection in the bank vole. We propose to further investigate the functional significance of the identified genes, e.g., through common garden experiments and involving gene expression analysis, as they represent good candidates for local adaptation. Future studies should also look for spatial variation in physiological traits related to energy homeostasis or the immune system to ultimately link genetic variation, organismal physiology, and fitness traits in locally adapted populations.

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Contributions

Rf, MH, JAE conceived the study, NC, HH, MH, OH, PK, EM, OP, ADS contributed to provide samples. RF conducted laboratory analysis and bioinformatics under supervision of MH and JLAP, and RF drafted the manuscript as part of his PhD supervised by JAE and MH. JAE, JLAP and MH read and commented on several earlier versions. All authors read and improved the joint manuscript.

Keywords

Climate gradient, genomic analysis, *Clethrinomys glareolus* , local adaptations, rodent

Simplified story

Many species are distributed across climatic gradients and are expected to adapt locally to climatic conditions such as temperature or precipitation. Here, we investigate genomic adaptations in a small mammal distributed throughout Europe (3,200 km) using a multivariate and multimethod approach, sampling 12 populations and 275 individuals. We found strong spatial structuring of populations, and identified candidate genes for climate adaptation. Signatures of environmental selection were associated with genes mainly related to energy homeostasis and pathogen response. Understanding local adaptation is important for our ability to predict species resilience to climate change.

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Data Accessibility Statement

Genetic data:

Raw read, SNP genotypes, input files for analyses, code and other relevant files will be deposited in the Dryad Digital Repository upon acceptance

Sample metadata:

Metadata will be stored in the Dryad Digital Repository upon acceptance

Benefit-Sharing Statement

Benefits Generated: A research collaboration was developed with scientists from the countries providing genetic samples, all collaborators are included as co-authors, the results of research have been shared with the provider communities and the broader scientific community (see above), and the research addresses a priority concern, in this case the adaptations of mammals to rising temperatures