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Research Article

Signatures of differential local adaptation indicate climate matching with specific native-range habitats in invasive raccoons (*Procyon lotor*)

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Abstract

Invasive species pose a major threat to ecosystems through habitat destruction, disease transmission and competition for resources. Invasion success depends on the adaptive potential (i.e. the ability to respond to selection pressures that is dependent on the levels of genetic variation) of the invading species. Here, we used reduced representation sequencing of native and European invasive raccoon populations to identify putatively adaptive changes across geographic regions. Our findings indicate that invasive raccoon populations experienced a genome-wide reduction in genetic diversity; however, we detected putative signatures of selection that could be related to local adaptation within the invasive range. Analysis of candidate loci, associated with either native or invasive populations, revealed putative parallel selection pressures between the invasive range and one of the native populations, all inhabiting similar climatic and habitat niches. In contrast, the native population from a different climatic region experienced distinct selective pressures. These results highlight the importance of habitat similarity in invasion processes. They demonstrate that, when predicting the potential range of an invasive species, it is not only the geographic distribution of the native range that should be considered, but also the specific characteristics of the habitat. Candidate genomic regions were linked to traits that promote rapid expansion, including reproduction, cognitive function, stress response, immunity and energy production. Overall, this study illuminates how contemporary selection in raccoons may accelerate the invasion process.

Key words: Adaptation, biological invasions, climate matching, invasive range, native range, raccoon

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Introduction

Since the Industrial Revolution, the global transportation of species has been increasing, leading to shifts in species ranges and the emergence of increasing numbers of invasive alien species (Early et al. 2016). They are ranked fifth amongst direct drivers of change in nature with the largest relative global impacts (Mack et al. 2000; Seebens et al. 2017; Brondizio et al. 2019; Pyšek et al. 2020), contributing to habitat destruction, disease transmission, competition for resources, potentially leading to displacement or even extinction of native species (Molnar et al. 2008). The suitability of the environment where a species is introduced can greatly enhance the probability of invasion, as alien species have a higher probability of

success if they are introduced into regions with a climate similar to that found in their native area (Abellán et al. 2017; Redding et al. 2019). When the habitat differs, the success of invasive species depends on their adaptive potential, i.e. their ability to respond to selection pressures. Species with a restricted adaptive potential have a lower likelihood of adapting to novel and changing environments (Lande and Shannon 1996; Merilä and Hendry 2014). Beyond individual plasticity within a species, the survival of organisms in new environments has been linked to the level of genomic diversity transferred to the novel range, allowing rapid adaptation (Dlugosch and Parker 2008; Rollins et al. 2013; Stange et al. 2021). Invasive species, despite often facing demographic bottlenecks and genetic drift, usually exhibit genetic diversity equal to or greater than what is found in the native range due to either the restoration of adaptive genetic diversity after the invasion event by admixture amongst populations from multiple introductions (Dlugosch et al. 2015) or by bringing substantial levels of adaptive genetic diversity from their native range (Uller and Leimu 2011; Lee 2016; Kołodziejczyk et al. 2025a).

The detailed insight into invasion demography and its effect on genetic diversity enables the separating of signals originating from neutral and selective processes. Combining multiple approaches to detect genomic signals of selection and comparing signals from putatively neutral and selective loci enables the identification of genomic changes associated with selection in the invasive range (Leigh et al. 2021; Sherpa and Després 2021). Studying the genomic footprint of selection in multiple invasive populations, as well as in native populations from two geographically distant regions, characterised by distinct climatic conditions, should enable the identification of specific genomic variants associated with either invasion success or adaptation to local environments.

Here, we used reduced representation sequencing of native and European invasive raccoon (*Procyon lotor*) populations to identify putatively adaptive changes amongst geographic regions. Raccoons are medium-sized, omnivorous mammals that inhabit forests and wetlands, but are also closely associated with urban habitats. They cope extremely well with diverse environmental conditions and often outcompete native species with similar ecology and life history traits (Jernelöv 2017). Native to North America, raccoons were introduced to Europe and now inhabit a majority of the continent proving their extreme invasive potential. Raccoons were first introduced to Germany in the 1930s, from where they have subsequently spread into neighbouring countries, including Poland where first populations were established in 1980s (Bartoszewicz et al. 2008; Biedrzycka et al. 2014). German populations, the largest in Europe, still serve as an important source of individuals for further spread (Hoekstra 1983; Biedrzycka et al. 2014; Maas et al. 2021). They were founded from at least four separate introductions, leaving a clear pattern in mitochondrial (Fischer et al. 2015) and microsatellite markers (Maas et al. 2021). In the countries of the former USSR, introductions occurred between 1936 and 1958 (Aliev and Sanderson 1966). Their present status is greatly unknown, but there is no strong continuity between the eastern and central European range. In France, at least three independent populations were established following introductions between 1966 and 2007 (Larroque et al. 2023). In Italy, the first known population was established in 2004 in the northern part of the country (Canova and Simone 2009), followed by the discovery of a population in central Italy established from individuals originating from zoological garden (Boscherini et al. 2020; Garofalo et al. 2024). In Spain, raccoons from at

least two separate introductions were first reported in 2003 and, within less than 10 years, were found in at least 28 localities (García et al. 2012), with the largest populations established around Madrid (Alda et al. 2013). Currently, raccoons have well established populations in the Netherlands, Belgium and Luxembourg (Maas et al. 2021) and almost all European countries (Stope 2019). The overview of raccoon introduction, spread and present status was summarised in several reviews (Lutz 1995; Jernelöv 2017; Salgado 2018; Stope 2019). The invasive raccoon populations in Europe descend from individuals originating from deliberate releases and escapes from fur farms, zoos and private owners, dating back to the beginning of 21st century (García et al. 2012; Boscherini et al. 2020; Larroque et al. 2023; Garofalo et al. 2024; van den Berge et al. 2025). Due to subsequent admixture of genetically divergent individuals originating from separate introduction events (Alda et al. 2013; Biedrzycka et al. 2014; Maas et al. 2021; Larroque et al. 2023), raccoons maintain relatively high overall genetic diversity. However, the relationship between neutral genetic diversity, adaptive potential and population fitness has been called into question, as neutral diversity may not reliably indicate a species' adaptive potential (Teixeira and Huber 2021). Comparing frequency shifts of potentially adaptive genetic variants between invasive populations and their native sources, while correcting for population structure, can provide evidence for local adaptation (Kawecki and Ebert 2004).

A recent study compared gut microbiomes of native and invasive raccoon populations and found no decline in richness or stability of microbiome fauna amongst invasive populations compared to what was found in native populations (Kołodziejczyk et al. 2025b), supporting raccoon adaptability across different environments. Moreover, Konopiński et al. (2023) revealed that the adaptive potential of invasive populations was retained in a set of immune-related loci. The raccoon's successful invasion across Europe is likely facilitated by species-specific traits with putative genetic underpinnings, including a high reproductive rate (Ritke 1990) and omnivorous diet that uses a broad range of available food resources ranging from plants (especially fruits) and invertebrates to waterfowl eggs and anthropogenic waste (Rulison et al. 2012). These characteristics may be associated with functional genomic diversity in regions associated to reproduction, immunity or dietary flexibility in both native and invasive populations.

This study sought to analyse raccoon populations from North America (i.e. the native range) and the central part of the invasive range in Europe to examine how populations in these regions might have evolved independently over the past 75 years. The structure of invasive raccoon populations has been well characterised, but the complex phylogeography (Cullingham et al. 2008) and the vast native range impair the identification of the exact source populations of the European invasion (Fischer et al. 2015). To mitigate this issue and to allow comparison of invasive populations' genetic diversity with native populations from both similar and different climatic conditions, we analysed two geographically and genetically distant native populations. In the invasive range, we analysed individuals from three areas: the invasion core, originating from separate introductions in Hessen, Saxony-Anhalt and Brandenburg (Germany); its eastern invasion front in western and central Poland; and an isolated population from Czechia, established independently in the early 2000s by individuals that escaped from zoos (Biedrzycka et al. 2019). We aimed to identify candidate genomic regions putatively associated with specific local adaptations in native and invasive populations. We expected to

find signals of selection in specific single nucleotide polymorphisms (SNPs) by comparing overall genomic diversity patterns with those found only at loci identified as outliers (i.e. showing significantly different variation patterns compared to the vast majority of putatively neutral SNP loci and, therefore, likely affected by selection forces). Additionally, we predicted that we would detect outlier loci other than those that had previously been detected in a study restricted only to SNPs located in immune–related genes suggesting that raccoon invasion success might be related selection on immunity (Konopiński et al. 2023). Second, we aimed to identify SNP loci that could point to putative adaptations associated with the raccoon invasion in Europe. The presence of such loci, exhibiting allele frequencies departing from neutral genetic background, could be linked to local selective pressures. Similar genetic diversity patterns amongst native and invasive populations inhabiting similar environments would point to parallel selective pressures, occurring before and after introduction to new range. Moreover, such a pattern may result from the fact that those native populations acted as a source of studied invasive populations and the detected pattern of selection occurred in the native range and was not erased by demographic processes associated with invasion. Genetic evidence putatively supporting the role of habitat similarities between native and invasive ranges are discussed (in this paper?) in relation to invasive species management and the evolutionary processes that lead to genomic differentiation amongst geographic regions of invasive species.

Materials and methods

Sample collection

Between 2021 and 2023, we collected a total of 162 tissue samples from raccoons from their native (near Melbourne, Florida (USA_F; n = 26) and between Lincoln and Syracuse, Nebraska (USA_N; n = 16)) and invasive range (Poland: The Warta Mouth National Park (PL_WM; n = 30) and Kampinos National Park (PL_K; n = 9), Germany: Brandenburg (GE_B; n = 13), Hessen (GE_H; n = 28) and Saxony-Anhalt (GE_S; n = 25) and Czechia (CZ; n = 15; Fig. 1). Samples from Florida were collected from adults transported to the Florida Wildlife Hospital, Inc. during euthanasia or surgical anaesthesia directly after a vehicle collision, while samples from Nebraska were collected as a result of local pest control programmes. Tissue samples from the invasive range were obtained during game activities in Poland, Germany and Czechia. All the samples were collected with sterile surgical blades and immediately stored in 96% ethanol. Upon arrival at the laboratory, samples were stored at -20 °C until DNA extraction.

DNA extraction and RADseq library preparation

DNA was extracted from skin and muscle tissues using NucleoSpin Tissue and NucleoSpin Tissue XS kit for DNA from cells and tissue (Macherey-Nagel) according to the manufacturer's protocol in the pre-PCR dedicated area. DNA was quantified using a Qubit Fluometer (ThermoFisher Scientific) and subsequently diluted to a final concentration of 20 ng/ul. Restriction site-Associated DNA (RAD) sequencing libraries were constructed following the Adapterama III protocol (Bayona-Vásquez et al. 2019) using PstI-HF, DdeI and NsiI enzymes (New England

Figure 1. Geographic locations of studied populations. Native populations (USA_N and USA_F) on the left side and invasive populations (GE_H, GE_B, GE_S, PL_WM, PL_K and CZ) on the right side.

Biolabs) together with relevant adapters indexed in accordance with design 3 from the aforementioned protocol. After digestion and ligation, all samples were pooled in equal volumes. At each nucleic acid purification step, short fragments and unincorporated adapters were removed using MagSi-NGS PREP Plus beads (magtivio) with a left-side selection procedure. After 12 cycles of PCR, libraries were visually verified via gel electrophoresis on a 1.5% agarose gel and quantified using a Qubit Fluometer. Size selection was performed using Pippin Prep (Sage Science) with the range of 400–600 bp. Prepared libraries were sent for sequencing as an external service by Novogene Ltd (UK) and sequenced using NovaSeq 6000 platform.

Bioinformatic processing

Raw fastq files were demultiplexed with process_radtags from the STACKS v.2 software package (Rochette et al. 2019). Individual reads were trimmed using TRIMMOMATIC v.0.39 (Bolger et al. 2014) with the following parameters: pair-end mode with a maximum of two mismatches, five bases cut at the start of each read, discarding reads shorter than 135 bp and applying a 3-base wide sliding window to trim when the average quality per base drops below 25. Reads were aligned to the reference genome (Plotor_platanus500, GCA_015708975.1; (Tsuchiya et al. 2021)) using Burrows-Wheeler Aligner (BWA) and MEM algorithm (Li 2013) with default parameters.

SNP calling and PCR duplicate removal were performed using gstacks, from the STACKS v.2 software package. The populations programme was used for initial SNP filtering and for generating input files for subsequent analyses. To minimise

false-positive SNPs, parameters were set to 0.5 for maximum observed heterozygosity and to 4 for the minimum minor allele count, meaning that at least four alleles are required for a SNP to be retained. Further filtering was conducted in VCFTOOLS v.0.1.16 (Danecek et al. 2011) with the following parameters: exclusion of genotypes with a quality score < 30 (--minGQ 30), a minimum depth of 10 (--minDP 10), retention of biallelic only (--min-alleles 2, --max-alleles 2) and a missing data threshold of 0.05 (using --max-missing 0.95, which ensures only loci with 95% called genotypes pass the filter) and a minor allele frequency greater than or equal to 0.01 (--maf 0.01). This dataset was used for outlier detection and selection scans. For population structure analyses (ADMIXTURE and PCA), we further pruned this dataset to ensure marker independence. First, we filtered out loci with a minor allele frequency lower than 0.05 (--maf 0.05) and SNPs that violated HWE (--hwe 1e-6). We used PLINK v.2.0.0-a.6.32LM (Chang et al. 2015) to identify loci in high linkage disequilibrium ($r^2 > 0.4$ within 50 kb windows). To account for population-specific linkage patterns, we calculated LD within each population independently. A consensus list of linked loci - defined as those appearing in high LD in at least two separate populations - was generated. From each linked pair, one SNP was randomly removed to reduce redundancy, while minimising data loss. The final exclusion was performed using BCFTOOLS (Li 2011).

Population structure analysis

To generate baseline characteristics of genetic differentiation, we used two different methods to resolve patterns of population structure. First, we used ADMIXTURE (Alexander et al. 2009), a model-based clustering method, evaluating clustering solutions for K ranging from 1–9 with 20 runs per each K value. The optimal number of clusters and replicate was determined by identifying the minimum cross-validation (CV) error. Results were visualised using ggplot2 in R v.4.4.2 (R Core Team 2024; Suppl. material 5: fig. S1). We then performed a Principal Components Analysis (PCA) using the glPCA function from the adegenet package in R (Jombart 2008). Missing data were replaced with mean allele frequencies following the recommendation of the package documentation (Jombart and Kamvar 2007).

Genetic diversity

To assess the level of genetic diversity in each cluster, diversity statistics, i.e. observed (H_O) and expected heterozygosity (H_E) and heterozygosity deficit (F_{IS}) along with their 95% confidence intervals obtained with 9999 bootstrap replicates, were calculated using gl.report.heterozygosity and the number of private alleles (*PA*) was calculated using gl.report.pa functions from dartR R package (Gruber et al. 2018). Percent of polymorphic loci (*PPL*) in each population was calculated using the populations programme in Stacks v.2 (Rochette et al. 2019). Nucleotide diversity (π) and Tajima's *D* were calculated using a custom script in R available at https://github.com/konopinski/tajima/. To account for differences in sample number between clusters, allelic richness (*AR*) was calculated using the allelic.richness function in the hierfstat R package (Goudet and Jombart 2004). To check whether differences in diversity statistics between native (USA) and invasive (Europe) ranges were significant, a linear mixed-effects model analysis was performed using the lme4 package in R (Bates et al. 2015).

Each of the diversity statistics was set as the response variable, range (native vs. invasive) as a fixed effect and loci as a random effect. Afterwards, we performed a test comparing the full and null models to assess whether the range had a significant effect on diversity statistics.

Patterns of selection

We aimed to differentiate processes affecting genomic diversity that were primarily shaped by demographic history from those influenced by local selection by identifying outlier SNPs and comparing diversity between outlier and neutral loci. Demographic history strongly influences spatial genetic differentiation and may blur the effect of selection. Processes such as allele surfing, causing rapid drift of some alleles at the expanding population front, hybridisation between divergent populations or spatial autocorrelation established during expansion from the introduction site may resemble patterns of local adaptation (Hoban et al. 2016). Still, alleles involved in local adaptation should show greater than average genetic differentiation amongst populations (Lewontin and Krakauer 1973; Beaumont 2005). Detection of such outliers can be plausible when analysing high number of loci (Tiffin and Ross-Ibarra 2014). Although demographic null models accounting for complex demographic scenarios, such as admixture, have not been developed, complementary use of approaches that are based on different models of allele frequency distribution allows for strong reduction of false positives (Lotterhos et al. 2018). First, we used BAYESCAN (Foll 2012), that uses Dirichlet distribution of allele frequencies and employs wide range of demographic models, including unequal migration. It identifies differences in allele frequencies by comparing each population's allele frequency to the common gene pool, using population-specific F_{ST} to highlight loci with outlying signals. Posterior odds in BAYESCAN were set to 1 to reduce false positives. Second, we used the pcadapt R package to detect loci under selection by identifying outliers from the pattern observed in principal component analysis (Luu et al. 2017). PCADAPT identifies outliers along different axes of structure and allows spatial covariance to be calculated from subset of SNPs. False positives were filtered using Benjamini-Hochberg correction as implemented in the qvalue function from the qvalue R package (Storey et al. 2025). We then adopted a pooled approach, classifying a SNP as an outlier for further analyses only if it was identified by both methods.

Since both BAYESCAN and PCADAPT detect global outliers, they do not identify specific populations or population groups where selection occurs. Therefore, we calculated the C_2-contrast statistic using BAYPASS (Gautier 2015) to detect outliers between geographic ranges. This method accounts for the neutral evolutionary history of populations by incorporating the scaled covariance matrix of the population allele frequencies (Ω matrix) and the ancestral (across population) allele frequency (Olazcuaga et al. 2020). These components enable the identification of candidate SNPs potentially associated with invasion status, providing a more targeted approach to detect selection patterns specific to range differentiation (Olazcuaga et al. 2020). As a representation of loci evolving neutrally, we used SNPs located at four-fold degenerate sites. Since all changes at the third position of such codons do not alter amino acids, evolution in these sites is considered a strong approximation of neutral expectations (Künstner et al. 2011).

To reveal diversity patterns within outlier loci, we calculated nucleotide diversity (π) and Tajima's D estimates using a custom R script (https://github.com/konopinski/tajima/) and compared the diversity patterns of outlier SNPs with those of neutral SNPs. While outlier loci are calculated using between-population differences in allele frequencies, Tajima's D and π estimates can prove helpful in an interpretation of forces shaping diversity in specific loci within population using site frequency spectrum and diversity at the specific loci. Tajima's D values oscillating around zero are suggesting neutral evolution, while $D > 1$ coupled with high nucleotide diversity may be suggestive of balancing selection. In contrast, loci with $D < -1$ and low π may be indicative of positive selection. Similar patterns can be created also by demographic processes, such as population expansion or contraction. Nevertheless, comparing patterns of diversity between outlier and neutral loci can give additional insight into processes shaping their diversity. Both diversity metrics were compared between: 1) the neutral SNP dataset and the global outliers and 2) the neutral SNP dataset and contrast outliers for each populations pair. The significance was assessed with linear mixed-effect models, with Tajima's D and π estimates as response variables, populations as fixed effects and loci as random effects using the lme4 package (Bates et al. 2015) and emmeans package in R (Lenth and Piaskowski 2017).

Lastly, we attempted to explore the potential role of genes subjected to selection in the invasion success. We used SNPEFF (Cingolani et al. 2012) to annotate putative outliers, determine their location in genome (i.e. the location of scaffolds containing outlier SNPs) and their position within genes (whether they are in coding, non-coding or intergenic regions). Genes associated with outlier SNPs were grouped into three main gene ontology (GO) domains: Biological Processes (BP), Molecular Functions (MF) and Cellular Components (CC). Gene enrichment analysis was performed using the topGO R package and using the Fisher's exact test and applying two algorithms: classic (the significance is tested independently, dismissing terms related in hierarchy (Bochalis et al. 2025)) and weight (each annotated gene is assigned to a weight, based on the neighbouring GO terms scores (Alexa et al. 2006)), followed by the p-value Benjamini-Hochberg false discovery rate (FDR) adjustment. Additionally, genes located on scaffolds containing outlier loci were identified using INTERPROSCAN (Jones et al. 2014).

Results

After filtering, the mean total number of reads per individual was 5,112,216, ranging from 1,409,295 to 23,811,072. The average depth of coverage was 92.5x, with a minimum of 25.4x and a maximum of 437.4x. The mean mapping success per sample was 97.14% ranging from 73.64% to 98.71%. Using STACKS pipeline, we initially obtained a dataset containing 5,200,166 SNPs. After initial filtering with the POPULATIONS programme with --max-obs-het 0.5 and --min-mac 4, the dataset was reduced to 2,512,954 sites. Applying quality filtering in VCFTOOLS (--minGQ 30, --minDP 10 and --mac 3 --max-missing 0.95) and limiting the dataset to biallelic SNPs resulted in 55,760 SNPs used for selection scans and site frequency analysis (Tajima's D and nucleotide diversity). Additional filtering to obtain dataset for population structure analyses (--maf 0.05, --hwe 1e-6 and ld pruning) produced the final dataset of 15,021 SNPs used for population structure analysis.

Population structure analysis

According to the analysis of cross-validation error in ADMIXTURE, the optimal number of clusters for the analysed dataset was K = 4 (Suppl. material 5: fig. S1, Fig. 2A). Individuals from the native population in Florida were assigned to cluster 3 (Florida) whereas individuals from Nebraska showed highest proportion of assignments to cluster 3 with addition of assignment to three other clusters. Cluster 1 was the primary assignment for all individuals from Czechia. Individuals from the other invasive populations were assigned to clusters 1, 2, 3 and 4, with all individuals from Hessen (Germany) unequivocally assigned to cluster 2, Polish populations (i.e. Warta Mouth National Park and Kampinos National Park) predominantly assigned to cluster 4 and the individuals in the two remaining populations (i.e. Brandenburg and Saxony-Anhalt, Germany) exhibiting mixed assignment to the same two clusters.

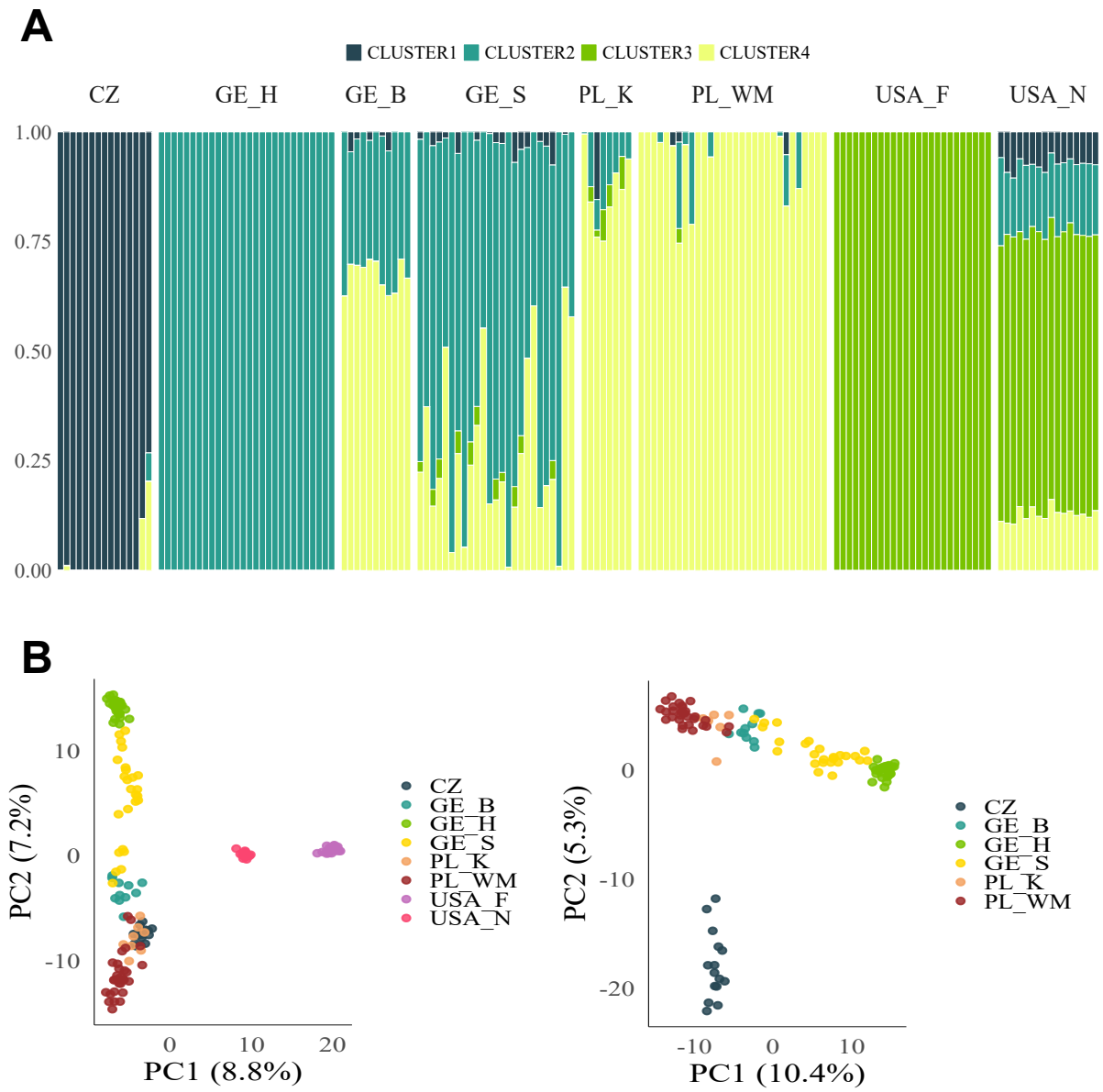

Figure 2. Clustering of the analysed individuals. **A.** Clustering based on ADMIXTURE results at K = 4; **B.** Clustering based on Principal component analysis (PCA). PCA plots are shown for all studied populations (left) and for invasive populations only (right).

Principal Component Analysis (PCA) revealed a similar pattern (Fig. 2B). Populations from the native range formed distinct clusters along PC1, with the Florida population showing the greatest separation from the invasive populations. Individuals from the invasive range were broadly distributed along the second principal component. When native populations were excluded from the analysis, the Czech population appeared the most differentiated amongst the invasive populations. The remaining individuals exhibited a spatially structured distribution, with the Polish (invasion front) and Hessen (invasion core) populations forming the outermost point clouds along the second principal component. The PCA results also suggested gene flow or individuals exchange between the Brandenburg and Saxony-Anhalt populations, consistent with their intermediate ancestry profiles in ADMIXTURE. As a result of both structure analyses, we combined the two Polish locations (PL_WM and PL_K) into one genetic population, hereafter called PL_WM+K. Nevertheless, although Polish and German populations form a continuum, mirroring raccoons' expansion towards east, we decided to analyse populations from Saxony-Anhalt (S) and Brandenburg (B) separately as their different origin was well proven in the previous studies (Fischer et al. 2015; Maas et al. 2021). The results presented here, involving a much higher number of loci, undoubtedly prove intensive admixture between two regions. Although PCA identified discrete clusters corresponding to the Czechia, Hessen, and Polish populations, it also indicated a gradient of genetic composition amongst invasive individuals, whereas more distinct clustering was observed in the native range.

Genetic diversity

Native populations exhibited significantly greater (linear mixed-effects model analysis; $p < 0.0001$, the table with results is included in Suppl. material 2) estimates of nucleotide diversity (π), allelic richness (*AR*) and observed (H_O) and expected heterozygosity (H_E) than those found in invasive populations. The lowest values of diversity were observed for CZ population (Table 1). These results indicated a general trend of higher genetic variation in native versus invasive populations. Furthermore, a number of private alleles in the native populations was an order of magnitude higher than in the invasive ones, except that populations PL_WM+K and GE_S F_{IS} values showed no significant departure from 0. The highest F_{IS} estimates were observed in GE_S, PL_WM+K and USA_F, indicating slight

Table 1. Genetic diversity estimates in invasive (CZ, GE_B, GE_S, GE_H and PL_WM+K) and native populations (USA_F and USA_N). Studied sites: 55,760. *Pop* - population, *N* - number of analysed samples, *PA* - number of private alleles, *PPL* – percentage of polymorphic loci, *N gen* - mean number of individuals genotyped per locus in population, H_O – observed heterozygosity, H_E – expected heterozygosity, *AR* – allelic richness, F_{IS} - inbreeding coefficient, π – nucleotide diversity. Values of F_{IS} significantly different from 0 were marked with asterisks.

Pop	N	PA	PPL	N gen	H_O	H_E	AR	F_{IS}	π
CZ	15	288	45.9	14.8	0.140	0.141	1.356	0.042	0.147
GE_B	13	11	49.9	10.1	0.158	0.153	1.399	0.015	0.161
GE_H	28	230	50.9	27.7	0.149	0.151	1.373	0.024	0.154
GE_S	25	144	63.3	24.7	0.158	0.168	1.432	0.065*	0.171
PL_WM+K	38	259	61.6	36.4	0.145	0.154	1.394	0.064*	0.156
USA_F	25	5992	70.3	24.6	0.180	0.186	1.486	0.042	0.190
USA_N	16	2209	70.7	15.7	0.182	0.181	1.487	0.023	0.187

heterozygote deficiency, which may be related to only moderate differentiation within these genetic clusters, likely owing to a lack of closed populations and due to high connectivity amongst raccoon populations (Table 1).

Patterns of selection

Global outliers

Using the BAYESCAN and PCADAPT outlier detection methods, we identified 3,848 outlier SNPs - 3,268 by BAYESCAN and 672 by PCADAPT with 92 SNPs overlap between the two sets. Only those 92 shared SNPs were subsequently used in downstream analyses.

The values of Tajima's *D* and π in the neutral dataset (i.e. 4-fold SNPs) were primarily centred around zero across all populations, providing a baseline reference for interpreting the outlier dataset (Fig. 3A). In the set of global outliers, native populations (USA_F and USA_N) exhibited higher Tajima's *D* estimates ($p < 0.0001$, Table 2) and greater π ($p < 0.0001$ and $p = 0.002$, respectively) in comparison with neutral dataset (Fig. 3B), with distributions shifted towards higher values.

In contrast, Tajima's *D* estimates for most of the global outliers in the invasive range exhibited similar distribution as in case of 4-fold degenerate SNPs, nevertheless the differences were significant in all, but CZ population (Fig. 3B, Table 2, Suppl. material 1). Similarly, π values of global outliers showed distribution resembling neutrality, but their means were significantly divergent from those observed in neutral dataset (Table 2, Suppl. material 1).

Table 2. Results of linear mixed-effect models of Tajima's *D* and nucleotide diversity (π) values between the neutral and global outliers datasets for invasive (CZ, GE_B, GE_S, GE_H and PL_WM+K) and native populations (USA_F and USA_N).

	Tajima's *D*				π			
Pop	**estimate**	**df**	**t.ratio**	**p.value**	**estimate**	**df**	**t.ratio**	**p.value**
CZ	-0.0744	3035	-0.933	0.3508	-0.0614	2283	-3.075	0.0021
GE_B	-0.1483	3035	-1.859	0.0630	-0.1115	2283	-5.586	< 0.0001
GE_H	-0.2730	3035	-3.422	0.0006	-0.1245	2283	-6.240	< 0.0001
GE_S	-0.2264	3035	-2.838	0.0045	-0.1096	2283	-5.491	< 0.0001
PL_WM+K	-0.2843	3035	-3.563	0.0004	-0.0943	2283	-4.724	< 0.0001
USA_F	0.7573	3035	9.493	< 0.0001	0.1560	2283	7.815	< 0.0001
USA_N	0.4406	3035	5.522	< 0.0001	0.0737	2283	3.693	0.0002

Table 3. Results of linear mixed-effect models of Tajima's *D* and nucleotide diversity (π) values between the neutral and contrast outliers datasets for invasive (CZ, GE_B, GE_S, GE_H and PL_WM+K) and native populations (USA_F and USA_N).

	Tajima's *D*				π			
Pop	**estimate**	**df**	**t.ratio**	**p.value**	**estimate**	**df**	**t.ratio**	**p.value**
CZ	-0.1580	3258	-2.097	0.0360	0.0451	2456	2.426	0.0153
GE_B	0.5721	3258	7.594	< 0.0001	0.1798	2456	9.684	< 0.0001
GE_H	0.6680	3258	8.866	< 0.0001	0.1726	2456	9.292	< 0.0001
GE_S	0.9189	3258	12.197	< 0.0001	0.1986	2456	10.694	< 0.0001
PL_WM+K	0.6633	3258	8.804	< 0.0001	0.1587	2456	8.545	< 0.0001
USA_F	0.0061	3258	0.082	0.9350	-0.0374	2456	-2.015	0.0439
USA_N	0.5711	3258	7.581	<0.0001	0.1393	2456	7.503	< 0.0001

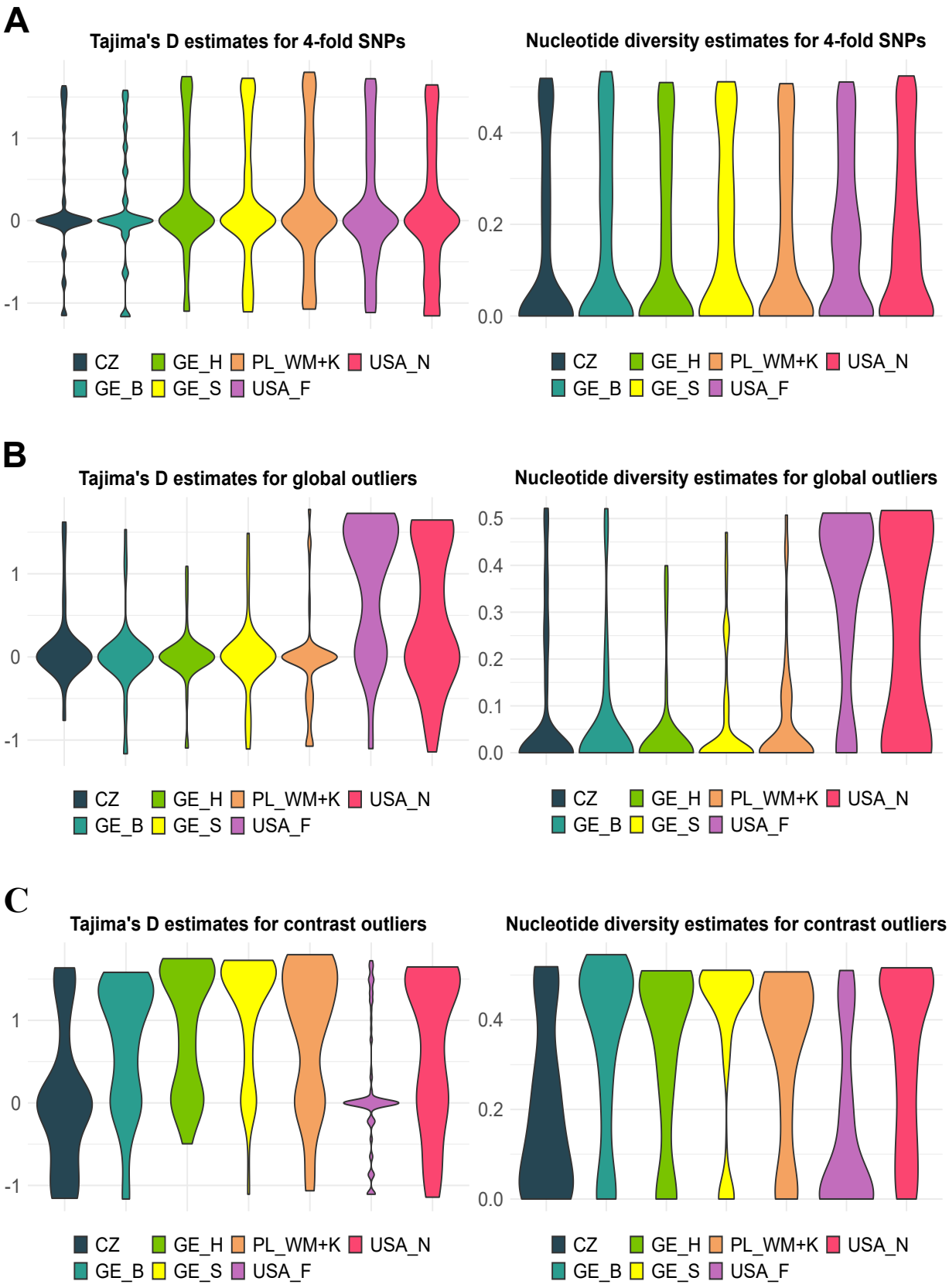

Figure 3. Distributions of Tajima's *D* (left) and nucleotide diversity (π; right). **A.** For 4-fold SNPs; **B.** For global outliers; **C.** For contrast outliers.

C2-contrast statistics - between ranges outliers

C2-contrast statistics were used to identify loci with outlying frequencies between ranges. The 113 outliers identified using the C2-contrast did not overlap with global outliers and exhibited a distinct pattern of Tajima's D and π estimates distribution (Fig. 3C). Contrast outliers for USA_F native population and CZ invasive population exhibited a clear pattern of neutral diversity in both Tajima's D and π estimates. In contrast, remaining invasive populations and native USA_N exhibited similar distribution of Tajima's D and π (Suppl. material 1). The majority of outliers were characterised by high values of both estimators (Fig. 3C). The mean values of Tajima's D estimates in contrast outliers were significantly higher in all, but USA_F populations, in comparison with neutral dataset (Table 3). The pattern observed for USA_F population is also visible in distinct allele frequencies of candidate SNPs. While the majority of contrast outliers present high allele frequencies in USA_N native population and invasive populations from Poland and Germany, candidate SNPs from USA_F present zero or close to zero values. On the other hand, candidate contrast outliers with low or zero frequencies in invasive and USA_N populations have high frequencies in USA_F population (Fig. 4, Suppl. material 1).

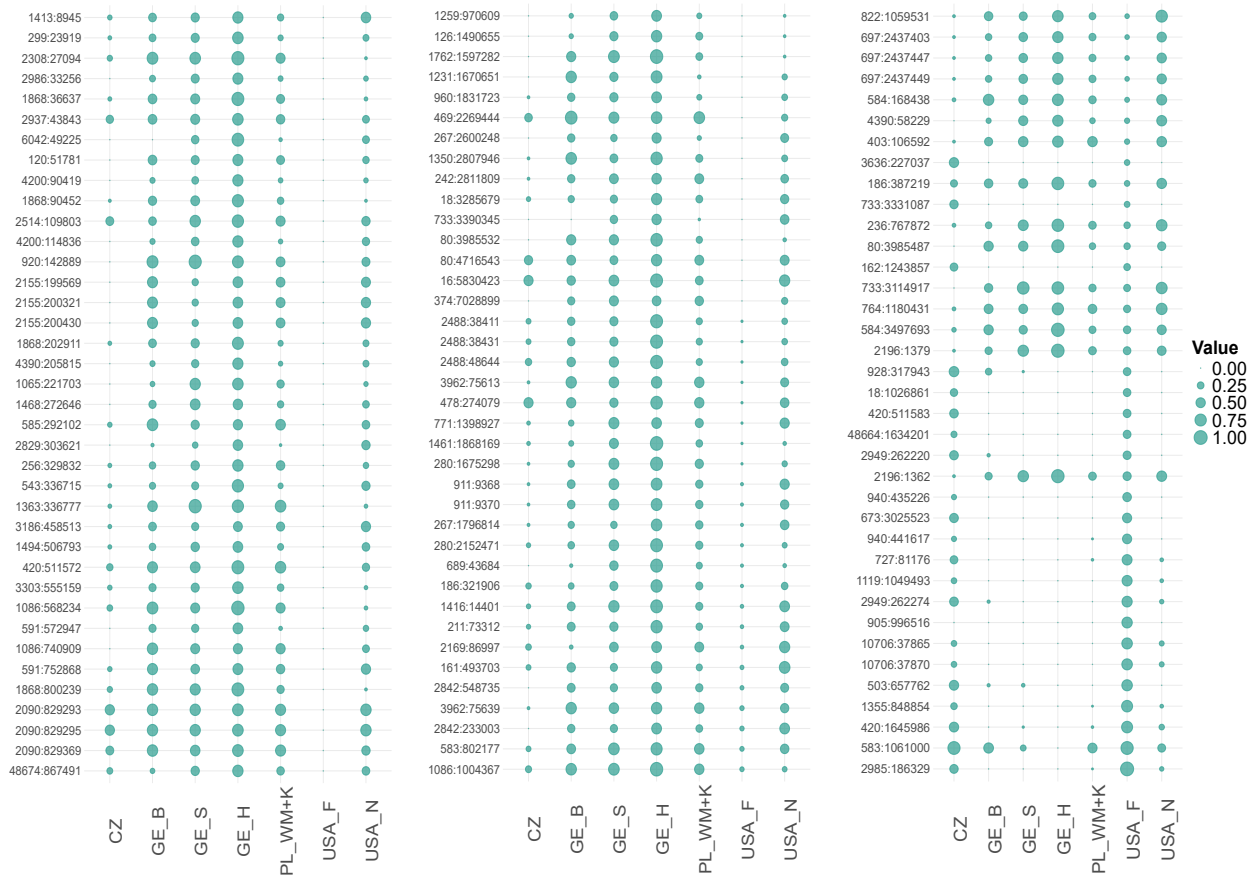

Figure 4. Allele frequencies of contrast outliers across all studied populations. IDs represent the abridged name of chromosome and position (CHR:POS) of SNPs according to the raccoon genome assembly Plotor_platanus500 (Tsuchiya et al. 2021). SNPs were ordered according to the USA_F allele frequency.

Genes/regions under selection

Out of the 92 global outlier SNPs, 34 were located in intergenic regions and were, therefore, excluded from gene enrichment analysis. The remaining 58 outlier SNPs included 39 with multiple predicted functional effects reflecting the presence of alternative gene models or overlapping transcripts. Amongst the SNP annotations, 40 were located upstream of genes (before the gene starts, often affecting regulation), 23 downstream of genes (after the transcription end region) and 46 in intronic regions (non-coding parts of genes). Additionally, two SNPs were synonymous variants (production of the same amino acid), one missense (production of different amino acid) and one was a stop-retained variant (change of the stop codon that could have functional consequences). The C2-contrast statistic identified 113 outlier SNPs differentiating the native and invasive ranges, of which 96 were located in intergenic regions and, therefore, excluded from gene enrichment analysis. Amongst the 113 remaining outlier SNPs, 67 were assigned to more than one predicted functional category. Of the SNPs located within or proximal to genes, intronic, downstream and upstream locations were predicted 105, 61 and 52 times, respectively; 14 synonymous, seven missense and four splice region variants were also predicted.

Amongst all global outlier SNPs, 44 were located in genes involved in immune response, cognitive functions, tissue repair and stress response processes potentially contributing to local adaptation (Suppl. material 4: table S1). For the C2-contrast results, over one hundred genes were identified, primarily associated with catalytic and enzymatic activity, development, microtubules arrangement, inflammation process, nervous system and odorant detection (Suppl. material 4: table S2).

GO enrichment analysis of global outliers revealed significantly enriched terms, primarily associated with genes related to reproduction and hormonal pathways (e.g. actin cytoskeletal organisation), intracellular metabolism (e.g. phosphatidylinositol dephosphorylation), protein and lipid binding functions, immune functions (e.g. scavenger receptor activity) and neurological features (e.g. synaptic cleft) (Suppl. material 5: fig. S2, for definitions see Suppl. material 3). For contrast outliers, significantly enriched GO terms showed patterns similar to those found within the global outlier dataset. Enriched terms were primarily related to the reproduction, development and hormonal pathways (e.g. regulation of hormone levels, sterol ester esterase activity and axoneme activity), muscle contraction and muscle tissue growth (e.g. microtubule-related), enzyme activity (e.g. glucokinase activity), signalling pathways (e.g. signalling receptor activity) and neurological features (e.g. related to spinal cord) (Suppl. material 5: fig. S3; for definitions, see Suppl. material 3).

Discussion

In this study, we analysed raccoons from native and invasive ranges to investigate genomic diversity, population structure and adaptation in a rapidly spreading invasive mammal. Rapid adaptation is likely one of the mechanisms providing resilience to new environments, enabling raccoons' expansion across Europe. Whether and to what extent, selection pressures are present in the invasive range depends on the level of habitat matching between native and invasive ranges (Abellán et al. 2017; Redding et al. 2019). Adaptation may appear from existing phenotypic plasticity (Merilä and Hendry 2014) reducing the need of selection acting on genetic variants. Still, the adaptive selection usually acts on the existing genetic variation

(Orr 2005). The level of genetic diversity, apart from the novel mutations, depends on the demographic history of the initial population (Santos et al. 2012). Our findings confirmed that invasive raccoon populations experienced a genome-wide reduction in genetic diversity. Despite this loss, we detected signatures of local adaptation that may be related to parallel climatic conditions within the invasive range and specific part of native distribution. In contrast, the native population from a different climatic region seems to experience distinct selective pressures. These results may point to the importance of habitat similarity in the invasion process. Candidate loci were located in genomic regions, associated with traits that could promote rapid expansion, including reproduction, cognitive function, stress response, immunity and energy production, suggesting that contemporary selection in raccoons may accelerate the invasion process.

Contrasting local patterns of selection

Allele frequencies are influenced by both demographic process and local selection (Willi et al. 2020). Comparing patterns across the genome in native and invasive ranges of a species and identifying loci that deviate from neutral expectations, can reveal adaptations to the new environment (Hoban et al. 2016; North et al. 2021; Hodgins et al. 2025). In contrast, the majority of loci evolve neutrally (King and Jukes 1969) and may be useful for identifying demographically driven population diversity and substructure. For example, genomic signatures of local adaptation related to an environmental stress response were detected in the tropical fruit fly, *Bactrocera tryoni*, a pest currently undergoing intensive expansion in Australia (Parvizi et al. 2024). Moreover, in an invasive population of Brown rat (*Rattus norvegicus*), outlier loci identified as under selection were linked to lipid metabolism and immune function, suggesting that these adaptations may have facilitated successful colonisation of new environments (Chen et al. 2021).

In this study, we first identified a set of outlier SNP loci, showing a significantly different pattern of between-population differentiation than the vast majority of SNPs evolving under neutral expectations. Then, we compared patterns of diversity between those two datasets. We observed a very different pattern of Tajima's *D* and nucleotide diversity between neutral and global outlier loci, but only for native populations. There was a positive shift towards higher values, resembling pattern of balancing selection acting on a substantial set of loci (balanced allele frequencies, high values of Tajima's *D* and nucleotide diversity; Figs 3A, B) in the native, but not in invasive range. This result suggests that the pattern of selection acting on specific loci in the native range was erased during establishment in the new range and associated demographic processes. Nevertheless, it has to be noted that the lack of knowledge on source populations and their allele frequencies prevents strong inference of balancing selection (Siewert and Voight 2017). In addition, short-term balancing selection is very difficult to detect because it only mildly affects the local patterns of variation (Fijarczyk and Babik 2015). One of the aims of this study was to identify SNPs located in genomic regions involved in adaptive processes related to the raccoon's invasion in Europe. To achieve this, we identified SNPs exhibiting contrasting allele frequencies between native and invasive ranges, while correcting for neutral evolutionary history. Using this approach, Olazcuaga et al. (2020) identified SNPs linked to the invasion success in *Drosophila suzukii* by analysing allele frequency contrasts, corrected for population structure. Similarly, Giska et al. (2022)

detected loci associated with environmental adaptations in mountain hares (*Lepus timidus*) across distinct habitats and Battlay et al. (2025) found loci under divergent selection between native and invasive ranges in *Ambrosia artemisiifolia*. Our study identified a large set of outlier SNPs potentially associated with divergence between ranges. However, detailed inspection of allele frequency distributions (Fig. 4) revealed that, for most SNPs, the contrast was caused by low or zero allele frequencies in the native Florida population, while high allele frequencies in native Nebraska and invasive populations from the continuous part of the invasion range (GE_H, GE_B, GE_S, PL_WM+K) or opposite – variants absent or in low frequencies in mentioned invasive populations and native Nebraska had high frequencies in Florida population (Fig. 4). The pattern could be due to parallel balancing selection acting on shared variants in Nebraska and invasive populations occupying similar habitats. Conversely, the same loci may be under directional selection in the Florida population, favouring specific alleles and resulting in extreme allele frequencies (near fixation). Although this pattern is visible in a small set of candidate loci, while the great majority of studied SNPs mirror population structure, without the knowledge of genetic diversity of populations that served as a source of studied invasive range, we cannot exclude the possibility that this pattern could be due to demographic factors, possibly closer genetic proximity between Nebraska and invasive range.

The identification of source populations and exhaustive sampling in both ranges would improve the precision of assessing allele frequency changes in relation to invasion (Olazcuaga et al. 2020). In the case of the raccoon invasion studied here, several factors complicate source population identification: the species' extensive native range, the absence of a comprehensive phylogeographic or population genetic study and extensive translocations of individuals across the native range (Cullingham et al. 2008). Additionally, in the European region, the raccoon population is formed from multiple independent introductions followed by subsequent admixture, further obscuring the geographic origins of the individuals that contributed to the European invasion and reinforcing the need for cautious interpretation of our findings.

To some extent, potential source populations can be inferred through ecological niche similarity analyses (Sherpa and Després 2021). Climate conditions, similar habitat structure, food availability or the extent of habitat urbanisation, matching between native and invaded ranges may facilitate rapid parallel adaptation, enabling invasive populations to re-establish adaptations to environmental characteristics present in their native habitats. In our study, the two native populations differ substantially in their habitat characteristics: Florida's subtropical and tropical climate is characterised by hot, humid summers and mild winters, whereas Nebraska's climate is characterised by mean monthly temperatures and rainfalls similar to those in central Europe (LaGrange 2005; Au 2018). Massive wetland ecosystems and pine forests of Nebraska may resemble the habitats occupied by the studied populations of raccoons (Nebraska Game and Parks Commission Publications 1991). All invasive populations inhabit either pine or mixed forest and wetland habitat or forest habitat in proximity to urbanised environment (Bartoszewicz et al. 2008; Jernelöv 2017; Salgado 2018). Local selective pressure and its effect on genetic diversity can be revealed by genetic-environmental associations (GEA) analysis. Unfortunately, our sampling scheme, especially in the native range, was insufficient to create the environmental gradient necessary to detect signatures of local adaptations (Lotterhos and Whitlock 2015). Moreover, expansion of invasive

population can generate allele frequency gradients similar to local adaptation associated with environmental characteristics. Repeated founder events and "allele surfing" at the invasion front (de Lafontaine et al. 2013) make identification of selection targets challenging; therefore, we are not able to distinguish with certainty between demographic and selective processes. Further, the pattern that resembles parallel selection may be also due to genetic proximity of invasive populations to their source in the native range that was maintained in a subset of loci, while erased by demographic process in others. The relatively smaller genetic distance between Nebraska and invasive populations, compared to that between Florida and the invasive range, is not itself sufficient to argue that Nebraska is the source of raccoon invasion in Europe. It is known that at least four independent introduction events were identified in central Germany (Fischer et al. 2015), but without thorough sampling of native range, it is impossible to identify the source of those initial populations. Still, similarities detected in loci putatively under selection may suggest that invasive raccoon populations may have originated from central USA. This finding suggest the importance of habitat matching in shaping adaptive responses and can be useful in planning invasive population management. Future studies incorporating broader sampling across the native range and additional sampling from the invasive range will be essential to further elucidate mechanisms underlying raccoon adaptation during invasion.

Enriched gene groups related to the raccoon's invasive potential

The GO terms enrichment analysis of the genes associated with global and contrast outlier SNPs revealed that the enriched gene groups are primarily related to reproduction, hormonal pathways, neurological processes, cognitive functions, the locomotor system and environmental-related responses. These enriched functions point to environmental drivers of divergence, but also may play a key role in facilitating raccoon invasion. Similar results have been recently obtained in studies of other mammalian invaders. For example, in invasive raccoon dogs (*Nyctereutes procyonoides*), enrichment was observed in genes related to energy metabolism, locomotor system, detoxification and lipid metabolism (Lan et al. 2022). Moreover, a study of the invasive brown rat (*Rattus norvegicus*) also identified the enriched gene groups involved metabolic functions, linking these findings to the success of species invasion (Chen et al. 2021). In both sets of outliers, we detected enrichment of GO terms related to reproduction. Globally, mammalian invasions are more likely in species with larger and more frequent litters and longer reproductive lifespans (Capellini et al. 2015). Indeed, high reproductive rates were shown in Japanese raccoon populations (Ikeda et al. 2004). The difference in selection pressures, revealed by contrast analysis, between native and invasive populations may reflect shifts in reproductive performance observed between the Florida population occupying sub-tropical habitat and remaining populations. We also observed enrichment in genes associated with neurological processes and various cognitive functions which can support neuroplasticity and, consequently, facilitate adaptation to a different environment (Axelrod et al. 2023). Cognitive capabilities, directly linked to nervous system function (Frasnelli 2021), have been shown to aid invasive species in locating and adopting new food sources and utilising a diversified diet (Griffin et al. 2022). Differences in cognitive function and problem-solving abilities have been documented between native red squirrels (*Sciurus vulgaris*) and invasive grey

squirrels (*Sciurus carolinensis*), with the invasive species demonstrating a competitive advantage (Chow et al. 2018). Selection on genes regulating ATP-dependent activity, in conjunction with those related to muscle contraction, appears central to changes in raccoon activity and exploratory behaviour, which may influence dispersal potential. Energetics and aerobic energy (ATP) production are amongst the most widely recognised physiological constraints on movement (Malishev and Kramer-Schadt 2021). The identification of enriched terms related to mobility gene groups that differentiate Nebraska and invasive populations from the Florida population suggests that mobility may vary depending on the habitat occupied.

Finally, the enriched GO terms were also related to environmental response. The enrichment in genes associated with mitochondrial cell respiration and oxidative stress response that we detected is usually linked to differential adaptation to cold and hot environments (White et al. 2011; Sokolova 2018; Wollenberg Valero et al. 2022). Further, the enrichment of functions associated with regulating the balance between immune response and the harmful effect of reactive oxygen species (DeCoursey 2013) could suggest an adaptive mechanism for mediating between immune activity and abiotic environmental pressure. The enrichment of GO terms related to the modulation of gene expression may reflect adaptive plasticity required for the colonisation of the invasive range (Mounger et al. 2021; Oh et al. 2022). Epigenetic mechanisms, particularly DNA methylation, are known to influence phenotypic plasticity (Carneiro and Lyko 2020). These mechanisms may facilitate environmental adaptation, as observed in invasive species such as salmon (Le Luyer et al. 2017) and the house sparrow (Liebl 2013; Sheldon et al. 2018).

Although we did not detect a direct overlap between SNPs identified as global and contrast outliers, the results of GO enrichment analysis show substantial overlap in enriched functions across both datasets (Suppl. material 3: figs S2, S3; for definitions, see Suppl. material 3). While we cannot pinpoint the exact genes or mechanisms involved in specific aspects of raccoon invasion, the outcomes of GO enrichment analysis suggest that SNPs under selection may play a role in local adaptation to the new range and in responses to novel environmental conditions. Interestingly, the results here seem to suggest that selection is likely acting on functional outcomes more than specific genetic alterations as the mechanism leading to local adaptive responses in the invasive range. Similarly, the study on native and invasive populations of the invasive weed *Ambrosia artemisiifolia* identified over-representation of genes involved in environmental response and pathogen defence and the signal of selection was detected independently by outlying SNP loci and gene copy number variation (Wilson et al. 2025). Moreover, a recent study of the microbiome of invasive raccoons revealed functional redundancy, where differing microbial communities maintained similar functional roles (Kołodziejczyk et al. 2025b). Likewise, although specific SNPs varied between global and contrast outlier methods, enriched functional categories remained conserved, suggesting a parallel pattern of functional stability amid genetic variation.

The history and current prospects of the raccoon invasion in Central Europe

We detected a clear pattern of genetic differentiation between native and invasive raccoon populations. Previous research has shown that European raccoon populations were established through at least four separate introductions (Fischer et

al. 2015; Maas et al. 2021) and the presence of distinct genetic clusters remains evident in our results. However, the relatively small genetic distances between invasive clusters suggest that, despite originating from independent introductions, the founding individuals were genetically more similar to each other than the two native populations analysed here. Most populations in the central part of raccoon invasive range belong to a continuum with an overlapping extent of the genetic clusters and, although traces of primary introduction events remain apparent in current genetic structure, the level of population admixture is substantial (Biedrzycka et al. 2014, 2019; Maas et al. 2021). Our analyses of population structure revealed connectivity between invasive populations that hindered clear population differentiation. The admixture between neighbouring invasive populations is also proved by significant F_{IS} value for PL_WM+K and GE_S populations. The only isolated cluster corresponds to the sampling site in Czechia. As shown in previous studies (Biedrzycka et al. 2014, 2019), this population is genetically isolated from other invasive populations, exhibiting lower genetic diversity and no signs of expansion. The population from Hessen in central Germany (GE_H), considered the primary introduction site in Europe in 1934, also formed a homogeneous cluster and exhibit reduced levels of diversity. According to the central-marginal hypothesis of species expansion, asymmetric, core-to-edge gene flow should be expected (Eckert et al. 2008). Here, this mechanism is supported by a proportion of assignment to the Hessian clade amongst genotypes in the population from eastern Germany (GE_B and GE_S) and genetic differentiation along the 2nd axis in PCA analysis (Fig. 2B) that is consistent with the geographical distribution of populations. Further, due to lowered habitat quality and higher population differentiation, lower genetic diversity may be expected across edge populations due to reduced population sizes, greater isolation and drift. Such a pattern was detected in invasive populations of invasive mealybug (Ma et al. 2020). In contrast to these expectations, we detected higher levels of admixture and marginally higher genetic diversity in raccoons from eastern Germany and Poland, despite the fact that the Polish population is currently located at the eastern front of raccoons' invasion from Germany. In the case of raccoons, the decreased habitat quality does not seem to be the case, as the species is known to thrive in a variety of habitats and use diverse food sources (Rulison et al. 2012). As a result, raccoons spread eastwards in high numbers, taking advantage of high habitat availability. The mixed support of the central-marginal hypothesis was also detected in the cane toad invasion in Australia (Trumbo et al. 2016), where the lack of a genetic diversity gradient was explained by habitat availability and connectivity. Although it is predicted that asymmetrical gene flow from the centre of a species' range may prevent adaptation of marginal populations and impact range expansion (Bridle and Vines 2007), the genetic diversity of raccoon marginal populations suggests that local adaptation should not be dismissed.

The very consistent levels of genetic diversity detected in the two native populations, despite large geographic distance and habitat dissimilarities between them, suggest that they are a good representation of native range diversity. Raccoons from Nebraska were more closely related to the European invasive populations, providing insight into the general North American region that likely served as the source for the European invasion. The intra-population diversity reflects patterns typically observed for invasive species. According to a systematic review of genomic invasion studies, over half of invasive populations experienced a reduction of

genetic diversity when compared to native populations, but, in most of all studied cases, the diversity was restored via admixture of divergent populations in the invasive range (Kołodziejczyk et al. 2025a). Our results also add support to this rule; although genetic variability within invasive populations was lower, they still maintained a high number of polymorphic SNP loci.

Conclusions and implications for invasion management

In summary, our findings demonstrate that the presumed signatures of local selection differ between native and invasive ranges and may be linked to different habitat characteristics. Candidate selection targets, located in genomic regions potentially linked to the raccoon's exceptional performance, appear more pronounced in native populations. Nevertheless, the pervasive influence of genetic drift has not entirely prevented local adaptation in invasive populations. The contrasting allele frequencies of candidate loci between populations inhabiting differentiated habitats, but not different ranges, suggest the role of habitat matching during invasion. Although the hypothesis on the role of habitat similarity between native and invasive ranges is central to explaining species invasions (Kolar and Lodge 2001), it is rarely confirmed by the analysis of genomic footprints of adaptation. Our study suggests that, when predicting the potential range of an invasive species, it is not only the geographic distribution of the native range that should be considered, but also the specific characteristics of the habitat. Conditions in the native range may pre-adapt populations for similar habitat types in the invaded range. The importance of habitat similarity in invasion processes, may be important even for generalist species such as the raccoon. Given that candidate loci were located in genomic regions involved in reproduction, cognitive function, the stress response, immunity and energy production, it is possible that those traits and functions facilitate rapid expansion. The raccoon is currently invading almost the entire European continent (Salgado 2018). It is predicted that this invasion process will continue. Eradication of the species is unlikely and management of the invasion mainly relies on protecting native species and fragile habitats (Cunze et al. 2025). While its broad climatic and habitat niche (Kochmann et al. 2021; Cunze et al. 2023) suggests it can invade a wide variety of habitats, our findings can be used to prioritise regions most vulnerable to invasion. The weakest selection signals detected in the least diverse and isolated invasive population (CZ) suggests that restricting gene flow between invasive populations may limit their adaptive potential. Future research involving population sampling along geographical gradients in both native and invasive ranges is needed to clarify associations of outlier loci with environmental characteristics and to determine the functional consequences of selection.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

J.K.: Methodology, Software, Formal analysis, Investigation, Data Curation, Writing – Original draft, Visualization; M.K.K.: Software, Formal analysis, Investigation, Writing – Review and Editing; E.A.H.: Investigation, Resources, Writing – Review and Editing; A.B.: Conceptualization, Methodology, Investigation, Resources, Writing – Original draft, Writing – Review and Editing, Supervision, Project administration, Funding Acquisition.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information. All raw DNA sequences have been deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under BioProject accession no. PRJNA1368613.

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Supplementary material 1

Outliers characteristics

Authors: Joanna Kołodziejczyk, Maciej K. Konopiński, Eric A. Hoffman, Aleksandra Biedrzycka

Data type: xlsx

Explanation note: Allele frequencies, Tajima's *D* and nucleotide diversity estimates for global and contrast outliers.

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Link: https://doi.org/10.3897/neobiota.106.182495.suppl1

Supplementary material 2

Results of LLM on diversity indices

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Data type: xlsx

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Link: https://doi.org/10.3897/neobiota.106.182495.suppl2

Supplementary material 3

GO Terms

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Data type: xlsx

Explanation note: GO Terms and definitions for genes in which global and contrast outliers were identified.

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Link: https://doi.org/10.3897/neobiota.106.182495.suppl3

Supplementary material 4

The list of genes and their functions

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Data type: pdf

Explanation note: Genes in which global and contrast outlier SNPs were identified and their functions.

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Supplementary material 5

Additional figures

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Data type: pdf

Explanation note: Cross-validation curve of K values prior to ADMIXTURE analysis, GO Enrichment Groups across outliers.

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