Genetyczne i pozagenetyczne mechanizmy kształtujące inwazyjność w świetle najnowszych badań – znaczenie lokalnych adaptacji i zmienności mikrobiomu na przykładzie szopa pracza *Procyon lotor*

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Kraków, 2025

Genetic and non-genetic mechanisms shaping invasiveness in light of the latest research – the importance of local adaptations and microbiome diversity using the example of the raccoon *Procyon lotor*

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Doctoral thesis

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Kraków, 2025

Table of contents

Streszczenie

Globalny rozwój transportu, handlu i turystyki przyczynia się do przenoszenia gatunków zwierząt oraz roślin poza ich rodzimy zasięg. Tylko niewielka część obcych gatunków zakłada populacje poza swoim rodzimym zasięgiem i rozprzestrzenia się, powodując poważne szkody zarówno ekologiczne, jak i ekonomiczne. Inwazyjne gatunki obce są jednym z najpoważniejszych problemów współczesnej ochrony przyrody, dlatego identyfikacja czynników, które przyczyniają się do sukcesu inwazji jest kluczowa dla zaplanowania działań zaradczych.

Celem mojej pracy doktorskiej było zbadanie grupy mechanizmów, które ułatwiają skuteczną adaptację, a w rezultacie umożliwiają inwazję. Skupiłam się na określeniu mechanizmów kształtujących zmienność genetyczną i działaniu doboru naturalnego oraz roli mikrobiomu jelitowego w przystosowaniu do nowego środowiska. Najnowsze badania podkreślają rolę danych genomowych w procesie zrozumienia mechanizmów tłumaczących sukces gatunków inwazyjnych. Uzupełnienie wyników badań genomowych analizą mikrobiomu jelitowego pozwoliło na lepsze zrozumienie czynników ułatwiających inwazję.

W pierwszej części mojej pracy, wykonałam systematyczny przegląd opublikowanych prac wykorzystujących dane genomowe do analizy wpływu mechanizmów demograficznych na kształtowanie się zmienności genetycznej oraz działanie doboru naturalnego w procesie inwazji. Zauważyłam wyraźny wzorzec ilościowy wskazujący, że pierwotny spadek zmienności spowodowany dryfem genetycznym oraz efektem wąskiego gardła, podczas zakładania populacji w nowym zasięgu, jest częsty, jednak nie powoduje trwałego obniżenia zmienności. Migracje pomiędzy genetycznie odmiennymi introdukowanymi populacjami oraz introgresja wariantów genetycznych w procesie hybrydyzacji powodują odtworzenie zmienności wewnątrzpopulacyjnej. Ponadto, ślady doboru naturalnego są najczęściej identyfikowane w regionach genomu związanych z funkcjami biologicznymi, które potencjalnie mogą ułatwiać inwazję, jak np. tolerancja na zmiany temperatury, odporność immunologiczna, szlaki metaboliczne oraz odporność na pestycydy. Systematyczny przegląd artykułów umożliwił określenie luk w wiedzy i zaproponowanie przyszłych kierunków badań.

Wzorzec, który zidentyfikowałam na podstawie analizy opublikowanych artykułów potwierdziłam badając strukturę genetyczną i zmiany adaptacyjne, związane z inwazją szopa pracza *Procyon lotor*, będącego jednym z najszybciej rozprzestrzeniających się inwazyjnych ssaków w Europie. Odnotowałam obniżenie zmienności genetycznej w zasięgu inwazyjnym, i jednocześnie admiksję między populacjami inwazyjnymi. Obecność doboru równoległego pomiędzy populacjami inwazyjnymi oraz populacją rodzimą, występującymi w podobnych warunkach klimatycznych, sugeruje specyficzne lokalne adaptacje. Wyniki podkreślają

znaczenie podobieństwa siedlisk między zasięgami rodzimymi oraz inwazyjnymi. Ślady doboru zlokalizowane były w regionach genomu związanych z rozrodem, szlakami hormonalnymi, procesami neurologicznymi i poznawczymi, układem ruchu oraz procesami biochemicznymi odpowiadającymi na zmiany środowiskowe. Wykrycie działania doboru naturalnego w tych specyficznych grupach genów sugeruje, że lokalne adaptacje mogą mieć znaczenie w wygrywaniu przez szopy konkurencji z rodzimymi gatunkami.

W ostatniej części mojej pracy, porównałam skład i funkcjonalność mikrobiomu jelitowego rodzimych i inwazyjnych populacji szopa pracza jako czynnika potencjalne mającego znaczenie dla jego inwazji. Populacje z obydwu zasięgów różniły się składem mikrobiomu, co mogło być spowodowane odmienną dietą lub zmianą składu taksonów bakteryjnych w wyniku przeniesienia poza naturalny zasięg i inwazji. Populacje z zasięgu inwazyjnego były bardziej zróżnicowane między sobą niż badane populacje rodzime. Jednocześnie duża różnorodność wewnątrzpopulacyjna nie potwierdziła wpływu efektu założyciela na poziom zmienności mikrobiomu jelitowego inwazyjnych populacji. Populacje rodzime miały mniej różnorodny mikrobiom, nie tylko w obrębie populacji, ale również bardzo zbliżony pomiędzy odległymi geograficznie lokalizacjami. Może to wskazywać na wysoki poziom stabilności mikrobiomu sugerujący jego odporność i rezyliencję. Z drugiej strony może to również odzwierciedlać ubogą, opartą na źródłach antropogenicznych dietę. Pomimo wykrytych różnic w składzie, profile funkcjonalne mikrobiomu jelitowego rodzimych i inwazyjnych populacji pokrywały się, co wskazuje na funkcjonalną redundancję, czyli nakładanie się funkcji pełnionych przez różne grupy bakterii. W przypadku szopa pracza, wysokie zróżnicowanie i niezaburzona funkcjonalność mikrobiomu jelitowego w populacjach inwazyjnych może być kolejnym czynnikiem ułatwiającym inwazję.

Zarządzanie gatunkami inwazyjnymi jest najbardziej skuteczne podczas początkowych etapów inwazji, kiedy działania zaradcze powinny być skupione na przeciwdziałaniu migracji oraz zakładaniu nowych populacji. Wyniki przeglądu systematycznego, jak i badania inwazji szopa pracza, wskazują na wpływ mieszania się odmiennych genetycznie populacji na sukces inwazji. Stąd też, na późniejszych etapach inwazji działania stają się mniej efektywne, ponieważ odbudowanie zmienności genetycznej potencjalnie ułatwia adaptację i sukces w nowym zasięgu. Przy użyciu danych o zmienności genetycznej wykazałam, że zgodność siedliska pomiędzy zasięgiem naturalnym i inwazyjnym ma znaczenie dla sukcesu w nowym zasięgu, dlatego warunki klimatyczne i cechy środowiska powinny być brane pod uwagę podczas ochrony zagrożonych terenów. Dane genomowe mogą usprawnić zarządzanie gatunkami inwazyjnymi, nie tylko poprzez odtworzenie historii inwazji, ale również oszacowanie potencjału adaptacyjnego. W konsekwencji możliwe będzie zaplanowanie dopasowanych do gatunku efektywnych działań.

Summary

In the era of intensive human movements across and between countries and continents, numerous species are intentionally and unintentionally transferred out of their native ranges. However, only a fraction of those species establish successful populations outside their native ranges, causing serious ecological and economic damage. Invasive alien species are one of the most serious concerns to modern nature conservation; therefore, a major focus is placed on identifying the traits that contribute to invasion success.

My PhD thesis aimed to investigate a group of mechanisms that drive successful adaptation and facilitate invasion. I focused on assessing mechanisms shaping genetic diversity and selection pressure, and the role of the gut microbiome in promoting local adaptations. Recent studies highlight the role of genomic data in providing a complex understanding of mechanisms underlying invasive species success. The complementation of the genomic findings with investigating the gut microbiomes offers an additional dimension to understanding factors facilitating invasions.

In the first part of my thesis, I conducted a systematic review of studies that employed genomic data to analyze the influence of demographic mechanisms shaping the genetic diversity and natural selection during invasions. I identified a clear pattern indicating that primary reductions of diversity caused by genetic drift and bottlenecks during establishment in the new range are frequent; however, they do not result in a permanent reduction of genomic diversity. The diversity is usually restored by admixture of individuals from separate introductions and/or introgression of advantageous genetic variants. Moreover, selection footprints are often detected in genomic regions related to specific biological functions, putatively facilitating invasion, such as temperature tolerance, immunity, metabolic pathways, and insecticide resistance. The systematic review of articles allowed for the detection of knowledge gaps and proposed directions for future research.

The pattern that I have identified by analysing published articles was confirmed by examining the genetic structure and adaptive changes related to the spread of the raccoon *Procyon lotor*, which is one of the most rapidly expanding invasive mammals in Europe. I detected the reduction of genetic diversity in the invasive range and admixture between invasive populations. The footprints of parallel selection between invasive populations and one from the native range, inhabiting similar environmental conditions, suggest habitat-specific local adaptations. The results highlight the importance of habitat matching between native and invasive ranges not only by environmental, but also by genetic data. Selection footprints were detected in regions of the genome related to reproduction, hormonal pathways, neurological processes, cognitive functions, locomotor system, and biochemical

processes associated with environmental changes. Selection footprints in those particular groups of genes suggest that local adaptations may play a role in outcompeting native species.

In the final part of my dissertation, I analyzed the differences in composition and functional profiles of raccoons' populations' gut microbiomes, as a non-genetic factor potentially influencing invasion. The microbiome composition of both ranges was differentiated, which may either reflect diverse diet sources or the loss and gain of microbial taxa during the invasion process. Invasive populations exhibited higher levels of inter-population microbiome diversity than the studied native populations. The high intra-population diversity did not confirm the influence of the population bottleneck on the level of microbiome diversity in invasive populations. Native populations, however, had a less diverse microbiome, similar between geographically distant locations, suggesting microbiome stability, indicating its resilience, but also possibly related to a more simplified, anthropogenic diet. Despite the differences in microbiome composition between ranges, the functional profiles of gut microbiomes from invasive and native populations overlapped, suggesting functional redundancy, i.e., the same functions are provided by different bacterial groups. In raccoons' invasive populations, the high level of differentiation and undisturbed functional profiles may be another factor facilitating invasion.

The management of invasive species is most effective during the initial stages of invasion, when actions to prevent the migrations and the establishment of new populations should be undertaken. Results of both systematic review and raccoons' invasion studies indicate that admixture of genetically divergent populations facilitates invasion. Therefore, during later invasion stages, countermeasures are less effective, as restoring the genetic diversity may drive local adaptations and invasion. Using the information on the genetic variation, I showed that habitat-matching between native and invasive range further facilitates success in the new range. Thus, climatic and environmental conditions should be considered while predicting vulnerability to invasion, even in such a cosmopolitan species as the raccoon. Genomic data may improve invasive species management, not only by reconstructing the invasion history, but also by estimating the adaptive potential. As a result, it will be possible to plan effective management actions tailored to particular species.

Introduction

In the era of intensive human movements across and between countries and continents, numerous species are intentionally and unintentionally transferred out of their native ranges. However, only a fraction of these species establish successful populations and outcompete native species. Those that do so represent a serious threat to ecosystems, being, next to habitat loss, one of the major drivers of species extinction (Molnar et al., 2008; Roy et al., 2024). Invasive alien species cause profound ecological impacts, ranging from habitat destruction, competition, predation, to pathogen transmission. Moreover, they can be a reason for substantial economic loss, including management costs and damage compensation in agriculture, forestry, fisheries, and the health-related sector (Bradley et al., 2024; Haubrock et al., 2021). A major focus of invasion ecology is to identify the traits contributing to invasion success. Understanding why some species become invasive while others do not, by uncovering the mechanisms underlying their success, is crucial for mitigating their harmful impact (Parker et al., 2013).

During the introduction, several factors facilitate successful adaptation. These include the availability of favourable habitat (Coulter et al., 2022), and the use of so-called novel weapons – traits that have not been present in the new range that enhance the competitiveness of invaders, e.g., allelopathy, new anti-herbivore, anti-fungal or anti-microbial effects. Furthermore, the invasion success may result from the availability of empty niches, which promote the faster spread and reduce competition. In ecosystems that have been disturbed by natural forces (e.g., destructive wildfires) or anthropogenic activity, the decreased abundance of native species creates unoccupied habitats for invaders (Daly et al., 2023). Other hypotheses include the release from natural enemies (predators or pathogens), allowing individuals to save energy otherwise spent on defence and thus increase fitness (Liu & Stiling, 2006), and the presence of a highly functional microbiome mediating essential phenotypic traits, e.g., energy metabolism, detoxification and disease resistance (Romeo et al., 2025). Despite the importance of the abovementioned characteristics, recent studies highlight that genomic data provide important insights into the mechanisms underlying invasion success. The research on mechanisms shaping population genetic diversity and molecular footprints of adaptation reveals how introduced populations overcome demographic bottlenecks, restore genetic diversity when adapting to novel habitats (Kaňuch et al., 2021; McGaughran et al., 2024). Next generation sequencing tools can also be employed to elucidate the role of the microbiome in facilitating invasion success (Romeo et al., 2025).

Adaptive potential, i.e. ability to respond to selection pressures, depends on the level of genetic diversity (Stange et al., 2021) and is crucial for the success of invasive species in novel environments (Lande & Shannon, 1996; Merilä & Hendry, 2014). It is believed that

invasive species often face a "genetic paradox". Although they are typically introduced in a limited number of individuals, leading to reductions of genetic diversity due to bottlenecks and genetic drift, they can adapt and succeed in novel environments. On the other hand, genetic diversity could be restored through admixture between different invasive populations or *via* hybridisation, enabling them to maintain high adaptive potential (Dlugosch et al., 2015; Estoup et al., 2016). In specific cases, invasive species may also retain substantial levels of adaptive genetic diversity from the native range (Lee, 2016). For example, frequent environmental fluctuations in the native range can promote balancing selection in source populations, maintaining multiple genetic variants that are brought to the new range during invasion. Genomic methods can be applied to the identification of the sources and pathways of invasion, estimating bottlenecks, and determining how pre-existing genetic variation in the native range influences invasive species. Genomics also offers tools for capturing footprints of novel adaptations occurring after introduction (North et al., 2021; Roe et al., 2019). Therefore, the number of studies using genomic methods to investigate invasion-related mechanisms has recently increased (McGaughran et al., 2024).

The application of population genetic theory to propose mechanisms shaping the genetic diversity of invasive species is well settled (Bock et al., 2015; Dlugosch et al., 2015; Dlugosch & Parker, 2008; McGaughran et al., 2024; North et al., 2021). Nevertheless, a comprehensive summary identifying those that contribute the most to the invasion success and are the most frequently reported has been lacking. To address this gap, as a part of my PhD dissertation, I conducted a systematic review of research articles employing genomic tools to study invasions. I searched for studies published since 2015, as the most recent reviews including genomic studies were published earlier (Bock et al., 2015; Chown et al., 2015). I quantified the frequency of different demographic processes related to invasion, mechanisms of selection, and genome features potentially related to invasion success. I identified gaps in knowledge and proposed future directions of research investigating the genetic mechanisms related to species invasions.

To place the conclusions drawn from the systematic review in the empirical context, the aim was to identify local adaptation footprints putatively enabling the extremely successful invasion of the raccoon *Procyon lotor* in Europe. Raccoons are native to North America and have become highly invasive in Europe, as well as in Japan. They inhabit forests and wetlands, but are also present in urban habitats (Jernelöv, 2017). Due to their high plasticity, high reproductive performance (Ritke, 1990) and diverse food resources (Rulison et al., 2012), raccoons adjust extremely well to changing environments and can outcompete native species, such as the European badger. In addition, they pose a threat through predation, food competition, aggression and pathogen transmission (Salgado, 2018). First known introductions

in Europe occurred in Germany about 80 years ago, as a result of the releases from fur farms and zoos, as well as for hunting and fauna enrichment purposes. At least four independent introduction events took place in Germany as revealed by the presence of separate genetic clusters and admixture of geographically nearby, yet genetically divergent populations (Fischer et al., 2015). The relatively high levels of genetic diversity (Biedrzycka et al., 2014, 2020) putatively facilitated rapid and extensive spread across Europe (Jernelöv, 2017).

Studying the genomic footprints of selection in several European invasive populations with different invasion histories, as well as in two native populations from geographically and climatically distant regions of the USA, is assumed to allow for the detection of specific genomic variants associated with invasion success or specific, local habitat-related adaptations.

To complement the findings on the role of genetic adaptation, I aimed to move beyond genetic factors potentially influencing invasion. A well-balanced gut microbiome is essential for maintaining individual fitness, through e.g., contributing to host plasticity (Kolodny & Schulenburg, 2020), supporting digestion (Henry et al., 2021) and stimulating immune response, thus protecting from pathogens (Chiu et al., 2017; Pickard et al., 2017). These functions can facilitate adaptation to new environments; however, the role of the microbiome in promoting invasion has been studied very rarely (but see Romeo et al., 2025). Invasive hosts may acquire new, potentially adaptive microbes from the local environment (Couch & Epps, 2022) or lose specific microbial taxa experiencing higher dispersion and dysbiosis that limits individual performance (Kohl, 2020; Zepeda-Paulo et al., 2018). These shifts do not necessarily translate into functional losses, as microbial functions may be maintained by different taxa (Louca et al., 2018). Importantly, this functional redundancy has not yet been explored in the context of invasive species.

By integrating multiple approaches, my dissertation enhances the understanding of adaptive mechanisms of invasive species and provides a significant contribution to the field of invasion biology.

Aims of the thesis

The presented research aimed to investigate the mechanisms underlying the successful adaptation of invasive species.

Aim 1: Assessing genetic mechanisms responsible for local adaptations, determining the response to environmental changes and invasion success.

a) Systematic review of studies that employed genomic tools to study invasion, with the focus on: estimating the frequency of demographic processes related to invasion, mechanisms of selection, and genome features potentially related to invasion success, as well as identifying gaps in knowledge and future directions of research on genetic mechanisms related to species invasions.

b) Characterization of genomic diversity and identification of local adaptation footprints promoting the extremely successful raccoon invasion in Europe, using SNP (single nucleotide polymorphism) loci. The invasion of raccoons in Europe served as a model to assess genetic mechanisms determining medium-sized mammal invasion success and to place results from the systematic review into an empirical context.

I hypothesized that:

The genetic composition of native and invasive populations differs because of:

1. Demographic processes associated with the establishment and expansion in the new range.

2. Natural selection acting in invasive populations as a response to local adaptation. The footprint of selection should be observed only in specific regions of the genome, while most of the diversity is evolving neutrally. Alternatively: 1) the strong impact of random demographic factors associated with establishment in the new range and subsequent expansion may prevent rapid adaptation to local conditions; 2) the lack of habitat differences between native and invasive ranges does not exert selective pressure.

Aim 2: Evaluating the importance of gut microbiome as a non-genetic factor, potentially influencing local adaptation, by assessing differences in gut microbiome between native and invasive populations.

I hypothesized that:

1. The gut microbiome composition of native and invasive populations differs because of demographic processes that shape the genetic differentiation of invasive populations are also reflected in the diversity of the microbiome.

2. The composition of a population's gut microbiome may enhance its adaptation in the new range. Alternatively, a higher degree of the gut microbiome dispersion in invasive populations, or the loss of important taxa, would indicate reduced microbiome functionality.

Article 1 – Genomic investigations of successful invasions: the picture emerging from recent studies

Kołodziejczyk, J., Fijarczyk, A., Porth, I., Robakowski, P., Vella, N., Vella, A., Kloch, A., & Biedrzycka, A. (2025). Genomic investigations of successful invasions: The picture emerging from recent studies. Biological Reviews, 100(3), 1396–1418. https://doi.org/10.1111/brv.70005

IF 2024 – 11.7

Points assigned to the journal by the Ministry of Education and Science – 200

Genomic investigations of successful invasions: the picture emerging from recent studies

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ABSTRACT

Invasion biology aims to identify traits and mechanisms that contribute to successful invasions, while also providing general insights into the mechanisms underlying population expansion and adaptation to rapid climate and habitat changes. Certain phenotypic attributes have been linked to successful invasions, and the role of genetics has been critical in understanding adaptation of invasive species. Nevertheless, a comprehensive summary evaluating the most common evolutionary mechanisms associated with successful invasions across species and environments is still lacking. Here we present a systematic review of studies since 2015 that have applied genomic tools to investigate mechanisms of successful invasions across different organisms. We examine demographic patterns such as changes in genomic diversity at the population level, the presence of genetic bottlenecks and gene flow in the invasive range. We review mechanisms of adaptation such as selection from standing genetic variation and *de novo* mutations, hybridisation and introgression, all of which can have an impact on invasion success. This comprehensive review of recent articles on the genomic diversity of invasive species led to the creation of a searchable database to provide researchers with an accessible resource. Analysis of this database allowed quantitative assessment of demographic and adaptive mechanisms acting in invasive species. A predominant role of admixture in increasing levels of genetic diversity enabling molecular adaptation in novel habitats is the most important finding of our study. The "genetic paradox" of invasive species was not validated in genomic data across species and ecosystems. Even though the presence of genetic drift and bottlenecks is commonly reported upon invasion, a large reduction in genomic diversity is rarely observed. Any decrease in genetic diversity is often relatively mild and almost always restored *via* gene flow between different invasive populations. The fact that loci under selection are frequently detected suggests that adaptation to novel habitats on a molecular level is not hindered. The above findings are confirmed herein for the first time in a semi-quantitative manner by molecular data. We also point to gaps and potential improvements in the design of studies of mechanisms driving rapid molecular adaptation in invasive populations. These include the scarcity of comprehensive studies that include sampling from multiple native and invasive populations, identification of invasion sources, longitudinal population sampling, and the integration of fitness measures into genomic analyses. We also note that the potential of whole genome studies is often not exploited fully in predicting invasive potential. Comparative genomic studies identifying genome features promoting invasions are underrepresented despite their potential for use as a tool in invasive species control.

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Key words: biological invasions, invasive species, adaptation, admixture, selection, next-generation sequencing, genomic diversity.

CONTENTS

I. INTRODUCTION

Present-day ecosystems are exposed to many threats from organisms that establish populations outside their natural range. While the transportation of live organisms around the world has increased dramatically since the beginning of the Industrial Revolution and is still on the rise (Bellard, Cassey & Blackburn, 2016; Zenetos & Galanidi, 2020), only some alien species become invasive (Mack *et al.*, 2000; Seebens *et al.*, 2017; Williamson & Fitter, 1996). According to the IUCN definition, invasive alien species are species that cause severe ecological or economic damage, with documented ecosystem impacts ranging from habitat destruction and disease transmission to displacement or even extinction of native species (Dueñas *et al.*, 2021; Fortič *et al.*, 2023; Molnar *et al.*, 2008). Therefore, a major focus in invasion biology is to identify mechanisms that contribute to invasion success (Fournier *et al.*, 2019; Parker *et al.*, 2013). Predicting which species are likely to become invaders before their introduction outside their native range has been a prime objective of invasion biology. The comparison of phenotypic features of native and invasive populations has enabled the identification of intraspecific differences in phenotypes that facilitate invasion success (Hiatt & Flory, 2020). Native and invasive phenotypes arise from the interaction between genotype

and environment in their respective populations and the impact of stochastic events. Therefore, examining phenotypic, genetic, and ecological differences across native and invaded environments is essential for understanding the mechanisms driving successful invasions.

In addition to the practical value of genetic studies for informing invasive species management, research uncovering invasive traits offers broader insights into the mechanisms of population expansion and adaptation to rapid climate and habitat change (Moran & Alexander, 2014). The most frequently cited hypotheses explaining invasion success highlight the roles of transport opportunity, propagule pressure, habitat matching, fecundity and population size as prerequisites and accelerators of species invasions. Invasive species often have significantly higher values for performance-related traits, better dispersal abilities, and shorter generation times compared to native species (Jeschke, 2014).

Species with low adaptive potential, that is a limited ability to respond to selection through phenotypic changes, face reduced chances of survival in new environments (Lande & Shannon, 1996; Merilä & Hendry, 2014). Adaptive potential and species ability to evolve depends on the level of genomic diversity (Day, 2015). While ecological factors are key determinants for invasion success, the role of genetics has been more challenging to demonstrate. Invasions are often

characterised by a "genetic paradox": despite reductions of genetic diversity during population establishment from a limited number of founding individuals, invasive species typically show high capability to adapt to novel conditions that sometimes allows them to outperform native species (Dlugosch *et al.*, 2015; Estoup *et al.*, 2016). This can be partially explained by reduced environmental pressure, for example resulting from release from natural enemies (Brian & Catford, 2023). On the other hand, admixture of phylogenetically divergent populations coming into contact in the invasive range can restore genetic diversity. Genomics of invasive species can be leveraged to identify populations of origin and invasion routes, and to estimate the timing of invasion and associated demographic bottlenecks, potentially identifying sources creating high levels of genetic diversity in invasive populations. Further, genomics can be employed to assess the role of pre-existing adaptation on facilitating invasion, and to identify the occurrence of *in situ* adaptations following invasion (North, McGaughran & Jiggins, 2021; Roe *et al.*, 2019). Balancing selection can facilitate maintenance of genetic variants important for adaptation to new conditions. New selective pressures can lead to selection of genetic variants acquired either before or after arrival in the invasive range. The latter can occur *via* admixture or introgression from other species or *via* mutations. Indeed, rapid adaptation is proposed to be crucial for the long-term success of invasive species (Dlugosch & Parker, 2008; Lee, 2002; Rollins *et al.*, 2013; Stern & Lee, 2020).

The importance of studying the genetics of biological invasions has been recognised for decades (Baker & Stebbins, 1965; Gray, 1986). During this period, numerous reviews have summarised the mechanisms that are prerequisites for creating and maintaining the levels of genetic diversity allowing for rapid adaptation and successful invasion. Despite many years of study, we still lack a comprehensive summary evaluating most common genetic mechanisms associated with successful invasion across species and environments. Crucial factors facilitating invasion at the level of genetic diversity were reviewed by Bock *et al.* (2015) and Dlugosch *et al.* (2015) before the genomics era. Technological and analytical advancements in the field of genomics have since transformed biological research and contributed significantly to unravelling the evolutionary basis of the success of invasive species (Jaspers *et al.*, 2021). The newest reviews referring to technological advances in studying the genomics of invasive species (McGaughran *et al.*, 2024; North *et al.*, 2021) present the current state of knowledge and future perspectives, but do not synthesise the numerous research findings to resolve the mechanisms involved in species invasions using genomic data. The number of articles utilising genomic methods to pinpoint or suggest different evolutionary mechanisms explaining the successful invasion of different species is growing rapidly.

Here we present a systematic review of studies that have accumulated since 2015 and that applied genomic tools to investigate mechanisms of successful invasions in fungi, plants, insects, and vertebrates. We address hypotheses related to mechanisms of species invasions and evaluate the frequency with which they appear in recent genomics literature. We focus on the main patterns associated with invasions, including changes in genetic diversity at the population level induced by demographic factors such as genetic bottlenecks or gene flow in the invasive range, as well as the evolutionary processes enabling adaptation, such as selection and adaptive introgression that are often related to invasion success. We examine the extent to which different invasion mechanisms have been studied across ecosystems. The objectives of this review include: (*i*) identifying the frequency of molecular footprints of adaptation in invasive populations and investigating whether specific selection events in the native species range are linked to their adaptation in the invasive range; (*ii*) determining the impact of demographic mechanisms that increase the levels of genomic diversity, such as admixture of divergent populations and interspecies hybridisation, in successful invasive populations; (*iii*) determining the impact of demographic mechanisms that reduce the levels of genomic diversity, such as genetic drift and bottlenecks, in invasive populations; (*iv*) identifying how often the levels of genetic diversity have been studied in the context of native populations and invasion routes; (*v*) analysing the genome characteristics associated with invasion success; and (*vi*) unravelling how often studies performed in a genetic context reported non-genetic factors as responsible for successful invasion.

As a result of this systematic review of articles on genomic diversity of invasive species, we also created a searchable database that can be used by future investigators.

II. METHODS

Our review followed a systematic review methodology according to the Collaboration for Environmental Evidence guidelines https://environmentalevidence.org/standards-table/. Published studies were identified by searching the *Web of Science* v.5.22.1 (https://www.webofscience.com/wos/) database with search years including articles published in 2015–2023. The specific search strings, the criteria for accepting articles and the methodology for extracting information for inclusion in our database, as well as a summary of the search is given as online Supporting Information in Appendix S1. The final collection of articles was assessed by reading the full text. While performing the text screening, we relied on the information and interpretation of results provided by the article authors. Therefore, the information that the genetic diversity was "high" or "low" or whether there was a decrease or increase in genetic diversity is based solely on the interpretation given in each article. Database S1 provides our full data set of all included articles, and the information collected from each article. Table 1 provides a glossary explaining population genetics terms in relation to species invasions.

Table 1. Glossary of population genetics terms used herein in relation to species invasions.

Term	Definition
Adaptive introgression	The transfer by introgression of relatively small genomic regions from a donor species that have positive fitness consequences in the recipient species. Contact between related, but previously isolated, species that occurs after invasion outside a species' natural range may result in adaptive introgression increasing the adaptive potential of invasive species.
Additive genetic variance	The total effect of loci measurably contributing to the trait.
Admixture	The process of mixing genetically distinct populations resulting in exchange of genetic variants between those populations. Multiple introductions of divergent invasive populations may result in increased overall genetic diversity.
Balancing selection	Natural selection that maintains genetic variation within a population by preserving multiple alleles at a particular locus. Refers to any type of selection that maintains genetic variance in a population, such as frequency-dependent selection, temporally or spatially fluctuating selection, and overdominance. Under appropriate conditions, temporally fluctuating selection could promote the accumulation and maintenance of genetic variation in the native range allowing faster adaptation in the invasive range.
Bottleneck	A sudden reduction in population size (demographic bottleneck), resulting in a reduction of genetic diversity (genetic bottleneck), occurring over one or few generations or a longer period. Bottleneck can act as a selective force when deleterious alleles are stochastically removed and the prevalence of adaptive alleles increases.
Bridgehead effect	Occurs when particular invasive population/populations are invasion sources for other invasive populations due to secondary introductions.
Directional selection	Natural selection where certain alleles are promoted, leading to diminishing genetic diversity in particular loci. Directional selection is expected to occur during population expansion into new environments as a result of response to novel environmental variables.
Genetic drift	Random change in allele frequencies following the colonization of a new range resulting in the loss of genetic diversity and random shifts in allele frequencies.
Effective population size	The number of individuals breeding in the population. Quantifies the magnitude of genetic drift and inbreeding in real-world populations. Range expansions during invasions should result reduced effective population size (N_e) due to strong effects of genetic drift.
Founder event	Establishment of a population from a small number of individuals resulting in a loss of genetic diversity and change in allele frequencies relative to the source population.
Genetic redundancy	A situation in which two or more genes encode a particular function. In the case of a deleterious mutation in one of those genes, the mutation effect on fitness is lower than expected.
Hard selective sweep	A pattern of genetic diversity when a single adaptive haplotype rises to high population frequency. Usually a result of a major effect mutation that arises on a single haplotype in a population and ultimately reaches fixation. As a consequence, the expected haplotype homozygosity surrounding the selected site is high.
Hybridization	Mating of individuals from different species or genetically distinct populations, leading to offspring with mixed genetic ancestries.
Introgression	An effect of hybridization when a foreign variant is permanently incorporated in the local gene pool through back-crossing.
Mitogenome	The mitochondrial genome.
Polygenic adaptation	A type of adaptation where many loci, each of small effect, contribute to the phenotype. Response to selection is caused by small frequency shifts of many alleles.
Selective sweep	Increase in frequency of favoured alleles caused by selection.
Soft selective sweep	A pattern of genetic diversity when multiple alleles at the same locus become favoured and their frequency increases. Can originate from standing genetic variation that becomes beneficial in a changing environment, or new recurrent *de novo* adaptive mutations. In the case of invasive populations, genetic variation carried from native range may become beneficial in the invasive range resulting in a soft sweep. Soft sweeps do not reduce genetic diversity around the beneficial mutation to the same extent as hard sweeps and may resemble patterns expected under neutrality.
Standing genetic variation	The genetic variation present in the population, as such constituting a source of variation on which selection can act more rapidly than on new mutations. In the case of invasive species is usually defined as genetic variation transferred from the native range.

III. SUMMARY OF COLLECTED STUDIES

(1) Taxonomy

Our search retrieved studies of species belonging to the kingdoms Animalia, Plantae and Fungi, with the highest proportion being animal species (Fig. 1A). Among these, the most represented phyla were Vertebrata and Arthropoda. Plant species were less represented, while only four studies focused on Fungi. Terrestrial animals were studied most frequently, followed by aquatic animals and terrestrial plants (Fig. 1B).
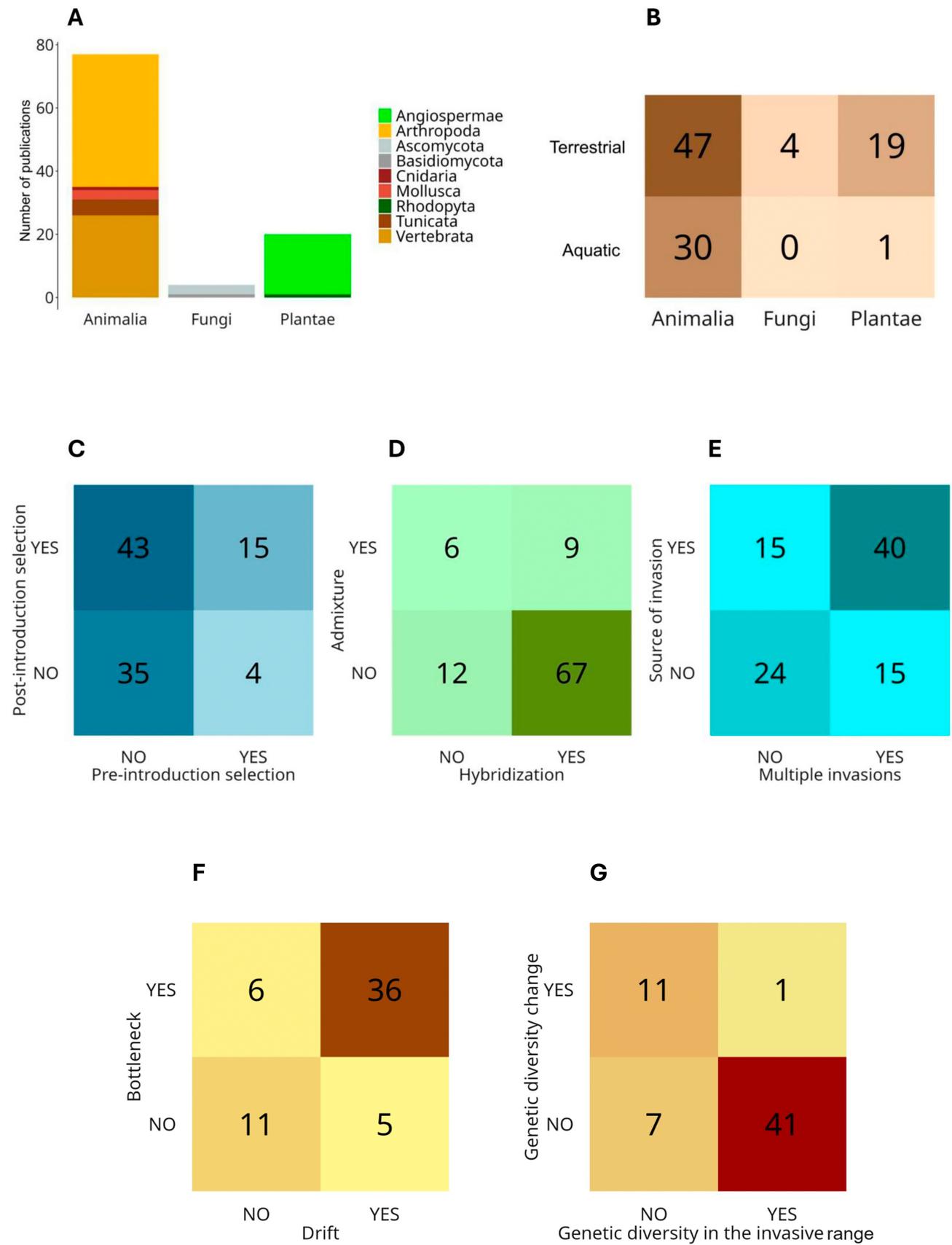
(Figure 1 legend continues on next page.)

(2) Types of studies

The search resulted in a database of 120 papers further classified into 101 "population studies" (i.e. analysing genetic diversity of invasive and/or native populations) and 19 genome, transcriptome, or genome and transcriptome analyses, which examined single/several genomes or transcriptomes of invasive species in the context of invasion. Twenty-four population studies also included genome/transcriptome analyses. Among the population studies, the most frequent topics investigated were selection processes in the invasive range and demographic events describing invasion (invasion sources and routes; number of invasions). Admixture between different invasive populations was more frequently a focus of study than hybridisation with other species and the majority of research related the analysis to native source populations (Fig. 1C–E).

(3) Applied methods

The sequencing methods used in a study determine the analytical approach that can be applied and the types of questions that can be addressed. Nearly half of the selected studies (57/120 studies) were based on reduced representation sequencing (RRS) methods. In population studies, 21/120 studies conducted whole genome sequencing (WGS) and nine/120 pooled sequencing (PoolSeq), with the remaining studies using less-common methods. Only 26/120 studies leveraged at least one whole genome sequence assembly, and five studies used transcriptome assembly for their analyses. The number of studies employing genomic methods has changed over time with a recent increase in the number of studies using WGS and RRS (Fig. 2).

IV. GENOMIC DIVERSITY CHANGE AND THE LEVEL OF DIVERSITY

(1) The level of genetic diversity: does genetic diversity decrease in invasive populations?

Previous reviews found contrasting trends in the levels of genetic diversity in invasive populations. These surveys analysing the genetic diversity of wild populations in an invasion context were performed before the genomic era, and therefore analysed studies mostly investigating a small number of traditional, presumably neutral markers

(allozymes, microsatellites, mitochondrial, etc.). Two such analyses found that 69% of invasive plants (Bossdorf *et al.*, 2005) and 63% of aquatic invaders (Roman & Darling, 2007) had levels of genetic diversity equal to or even greater than native populations. By contrast, Uller & Leimu (2011) reported moderate reductions in genetic diversity for the majority of invasive species. Using a limited number of neutral markers may lead to inadequate estimation of genetic diversity or missing information from ecologically relevant regions of the genome. A comprehensive assessment of genomic diversity is the most representative predictor of a species' evolutionary potential.

Out of the 101 population studies retrieved by our search, 62 analysed both native and invasive ranges. A comparison of genetic diversity levels in native and invasive ranges was provided in 51 studies out of these 62, but a statistical comparison of genomic diversity levels between source and invasive populations often was missing. There was, however, a clear pattern that statements of a reduction in genomic diversity in invasive populations are infrequent. We subdivided the studies into those stating that invasive populations show decreased genomic diversity (19/101) and those declaring that genetic diversity was maintained (here we combined articles reporting an increase, stable level or minor decrease of genetic diversity as stated by the authors; 43/101; Fig. 1G). In the remaining studies (39/101), no comparison of diversity levels between native and invasive ranges was performed, but in the majority of these, the level of diversity was indicated as high with only eight studies reporting low diversity in invasive populations. These findings are in line with previous results showing that the "genetic paradox" is not a general phenomenon in invasive species. Many studies did not assess genomic diversity levels at different invasion stages, with the majority performing sampling of native and invasive ranges at one time-point only. Sampling of genomic diversity across a time series in invasive ranges would allow assessment of diversity loss as a result of population establishment from a limited number of founders and to what extent it can be restored with time. Such data would also allow researchers to assess the speed of evolutionary changes at different invasion stages.

(2) The role of demographic factors: do invasive populations experience bottleneck/drift?

Despite the high levels of genetic diversity reported in the majority of studies (Fig. 1G), both genetic drift (detected in 47 of 101 studies), and bottlenecks (56 of 101 studies) were reported relatively frequently (Figs 1F, 3). Our approach

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(Figure legend continued from previous page.)
Fig. 1. (A) Number of publications identified by our search terms according to kingdom. (B) Number of publications in different kingdoms in terrestrial *versus* aquatic habitats. (C) Publications studying selection occurring pre- and post-introduction into the invasive range. (D) Publications studying admixture and hybridisation. (E) Publications studying source of invasion and the number of invasions. (F) Publications measuring genetic bottlenecks and/or genetic drift. (G) Publications assessing the change in genomic diversity between native and invasive ranges and/or quantifying the level of genomic diversity. Genomic diversity is categorised as: maintained = moderate decrease, no change, or increase in comparison with native populations; or decreased = marked decrease in comparison with native populations. Genomic diversity in the invasive range is categorised as: high = reported as high in the invasive range; or low = reported as low in the invasive range. Classification as high or low and maintained or decreased is based solely on the interpretation of the authors of each study.
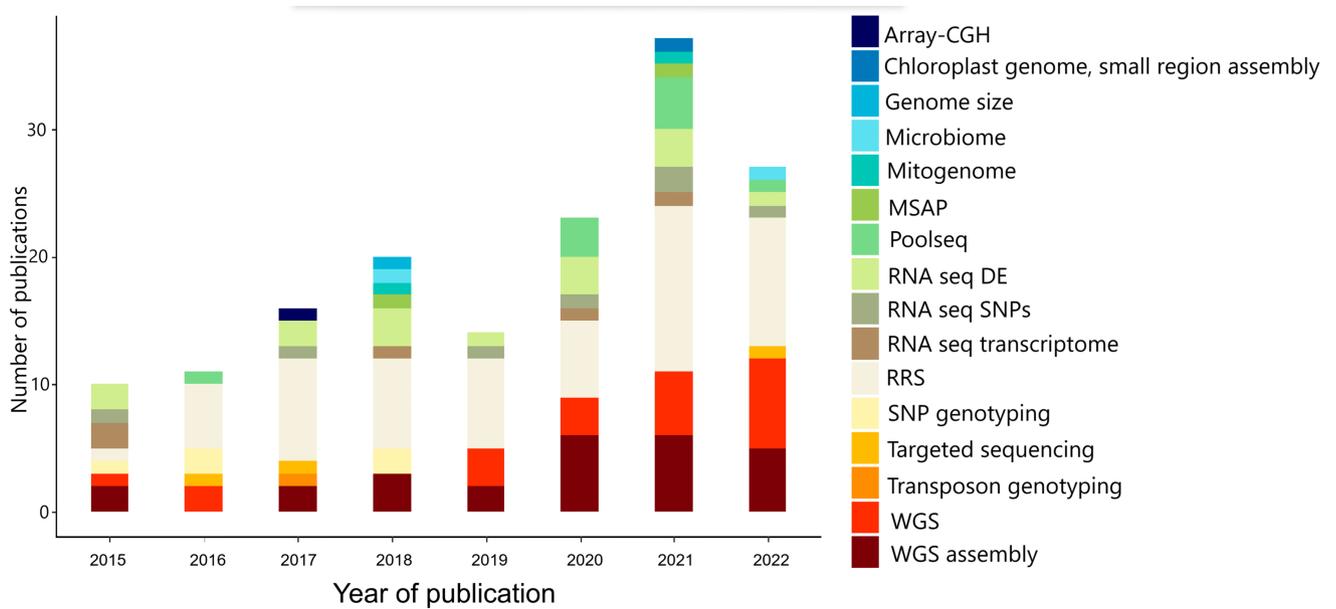
Fig. 2. Genomic methods applied in the studies included in our database for the period 2015–2022. CGH, comparative genetic hybridisation; DE, differential gene expression; MSAP, methylation sensitive amplified polymorphisms; RRS, reduced representation sequencing; SNP, single nucleotide polymorphism; WGS, whole genome sequencing.
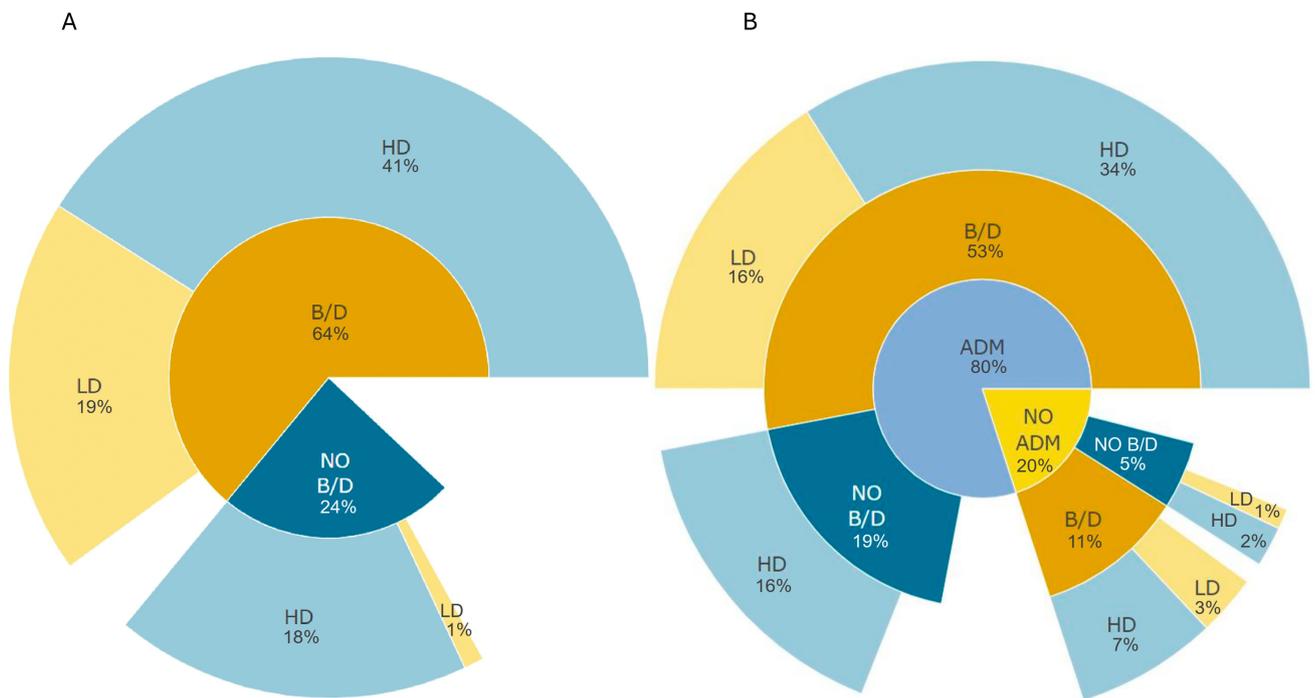

Fig. 3. Demographic factors shaping genetic diversity in invasive populations: (A) the percentage of articles detecting the presence of genetic bottleneck and/or genetic drift (both or one of the mechanisms was assessed in the article) or their absence (none of the mechanisms was confirmed in the article), and the percentage of articles where genetic diversity of invasive populations was assessed by the authors of each article as high or low; (B) the percentage of articles where admixture was reported or not, the percentage of articles detecting the presence of genetic bottleneck and/or genetic drift or not and the percentage of articles where genetic diversity of invasive populations was assessed by the article authors as high or low. Empty regions reflect a lack of information in the analysed articles. ADM, presence of admixture; B/D, presence of genetic bottleneck and/or genetic drift; HD, high level of genetic diversity in invasive populations; LD, low level of genetic diversity in invasive populations; NO ADM, absence of admixture; NO B/D, absence of genetic bottleneck and genetic drift.

relied on identifying specific key words when screening the text, and we note that some authors used one term but not the other. In most cases high levels of genetic diversity were restored by admixture between populations (Fig. 3B), recurrent invasions or the presence of bridgehead populations or introgression from other species. In several studies a lack of bottleneck signal was attributed to high propagule pressure during establishment in the invasive range (Chen *et al.*, 2021a; Goubert *et al.*, 2017) or to interspecific hybridisation before invasion (Burford Reiskind *et al.*, 2019; Popovic *et al.*, 2021). Bottlenecks can increase variation for quantitative traits by changing the relative magnitude of the genetic variances component, leading in some cases to an increase and allowing rapid evolutionary change in newly established populations (Turelli & Barton, 2006). Deleterious variants that have a higher probability of exposure in homozygotes after a population bottleneck can be purged more effectively from the population (Rius *et al.*, 2015). It has been suggested that bottlenecks can also act to increase additive genetic variance for traits with a non-additive genetic basis (Mularo, Bernal & DeWoody, 2022). Increased genetic variance resulting from a bottleneck was suggested in *Agarophyton vermiculophyllum* (Ohmi) populations (Flanagan *et al.*, 2021) that rapidly evolved greater tolerance to acute high temperatures and low salinities in the invasive range (Sotka *et al.*, 2018).

Although the occurrence of bottlenecks is frequently reported, the studies in our database lack time-series estimates that would enable precise detection of the timing of the event, the quantification of the impact of specific admixture events, and the interplay between these two factors.

(3) The role of admixture and introgression: do invasive populations experience gene flow?

Admixture between genetically divergent populations increases overall diversity and may foster genetic interactions among previously isolated alleles, allowing higher fitness of admixed populations and increasing the adaptive evolution of novel genotypes (Wagner *et al.*, 2017). The majority (61/80 studies studying admixture) demonstrated ongoing admixture at different stages of the invasion leading to increased genetic diversity (Fig. 3B). Only 20 out of all 101 population studies of studies reported no admixture and 19 out of 80 studies studying admixture reported a lack of significant change in the level of genomic diversity as an effect of admixture. Among these 19, high levels of diversity resulted from interspecific introgression or hybridisation in the native range. Therefore, an important finding of our survey is that admixture of invasive populations that substantially mitigates the effects of genetic drift and bottlenecks is extremely widespread (Figs 3B, 4). It appears that admixture of multiple invasive populations and, to some extent, interspecific introgression are the main mechanisms maintaining and increasing genomic diversity of invasive populations. Some studies have questioned the association between admixture and invasion success and its contribution to increased fitness of invaders (Chapple *et al.*, 2013; Dutech

et al., 2012; Wolfe, Blair & Penna, 2007). Nevertheless, the great majority of our surveyed studies identify admixture between populations as a significant process increasing genetic diversity and invasion success. One of the major unresolved questions in invasion genomics is whether, and to what extent, admixture is a cause or a result of successful establishment of invasive species, in particular when multiple invasions occur. These scenarios are non-mutually exclusive, but with careful study design or temporal sampling, genomics can be used to answer such questions. For example, population genomics can be used to infer the timing of admixture events, selective sweeps of introgressed genes, and demographic expansions, while experimental approaches, such as common garden experiments, can be used to compare the fitness of admixed *versus* non-admixed invaders.

(4) The role of mutation load on invasion success: do invasive populations carry a mutation load?

Further evidence for the lack of a "genetic paradox" is the absence of mutational load in invasive populations. Reduced effective population size during invasions may lead to inbreeding (Fauvergue *et al.*, 2012), which increases the homozygosity of segregating deleterious recessive alleles (i.e. genetic load) leading to a loss of fitness (i.e. inbreeding depression; Charlesworth & Willis, 2009). Additionally, in small populations, the frequency of deleterious alleles may increase stochastically (i.e. drift load), compromising fitness (Willi, Griffin & Van Buskirk, 2013). However, the genetic load in newly established invasive populations can be rapidly reduced by selection (Lacy & Ballou, 1998), admixture between populations from multiple introductions, or hybridisation in the new range. Rare deleterious alleles, usually present at low frequencies, can also be lost as a result of bottleneck upon introduction (Nei, Maruyama & Chakraborty, 1975). In fact, only two studies in our database suggested the possibility of genetic load in the genomic data. An analysis of the genomes of five Asteraceae species showed a greater proportion of deleterious alleles in the genome in the invasive range for one species, while the remaining species had a higher number of deleterious alleles in the native range (Hodgins *et al.*, 2015). The other study, analysing genome-wide diversity of two hybridising invasive species *Cakile edentula* and *Cakile maritima* predicted a greater fixation rate of weakly deleterious alleles in *C. edentula* due to its higher level of inbreeding (Rosinger *et al.*, 2021). The low number of recovered studies investigating genetic load reflects the rarity of whole-genome sequencing of large invasive populations coupled with fitness measurements.

V. GENOMIC FOOTPRINT OF ADAPTATION

(1) Preintroduction adaptation

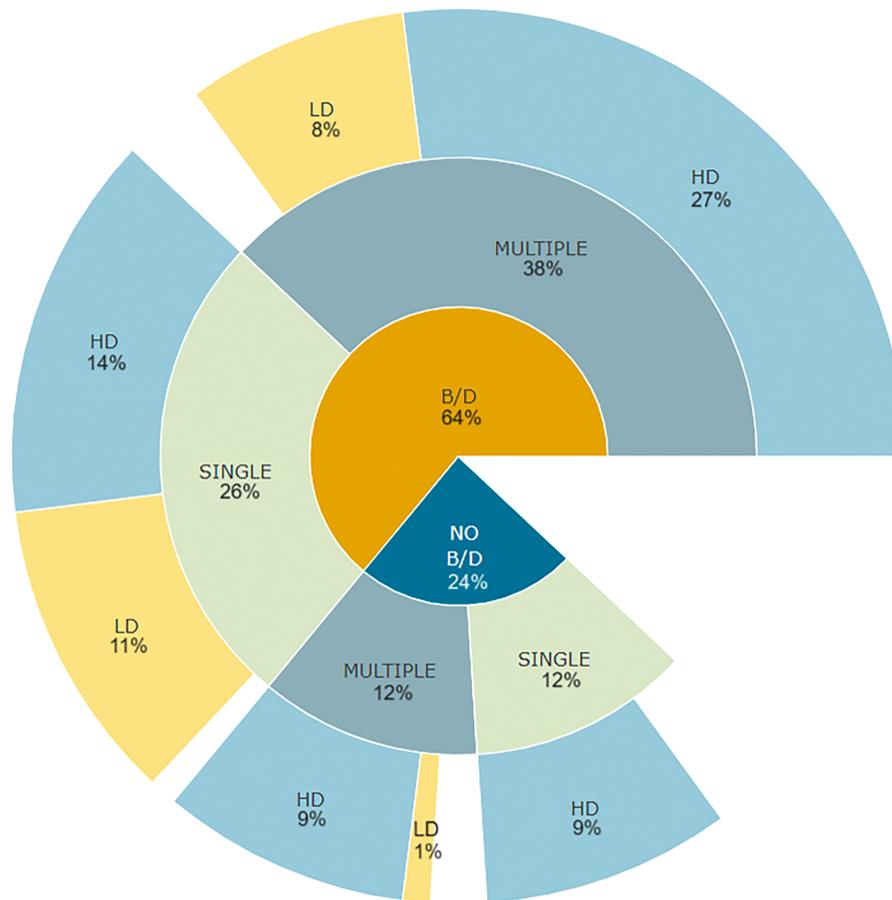One important consideration when analysing invasion success is the level of standing genetic variation available in

Fig. 4. Demographic factors shaping genetic diversity in invasive populations. Percentage of articles detecting the presence of genetic bottleneck and/or genetic drift (both or one of the mechanisms was assessed in the article) or its absence (none of the mechanisms was confirmed in the article), the percentage of articles where either single or multiple introductions were detected and the percentage of articles where genetic diversity of invasive populations was assessed by the authors of each article as high or low. Empty regions reflect a lack of information in the analysed articles. B/D, presence of genetic bottleneck and/or genetic drift; HD, high level of genetic diversity in invasive populations; LD, low level of genetic diversity in invasive populations; MULTIPLE, detection of more than one event of introduction; NO B/D, absence of genetic bottleneck and genetic drift; SINGLE, detection of one introduction.

invasive populations that can be leveraged during adaptation to novel habitats.

A fluctuating environment in the native range promotes the maintenance of multiple genetic variants (Lee & Gelembiuk, 2008). Diversity can in turn serve as a catalyst for selection in response to novel habitat conditions (Stern & Lee, 2020; Vera, Díez-del-Molino & García-Marín, 2016). Studies of genes shaped by balancing selection in both native and invasive habitats suggest that the retention of multiple variants of specific genes may be more important for survival of native and invasive populations than generally high levels of diversity across the genome (Vera *et al.*, 2016). This was shown to be the case for immune genes in native and invasive populations of the common raccoon (*Procyon lotor*) (Konopiński, Fijarczyk & Biedrzycka, 2023) where maintaining multiple variants provided protection from various pathogens.

High levels of adaptive genetic diversity in the invasive range can also be maintained by diversifying selection acting on the same loci as in the native range

(Burford Reiskind *et al.*, 2019; Goubert *et al.*, 2017; Krzemińska *et al.*, 2018). This special case of diversifying selection occurs when environmentally important traits have a polygenic background. Temperature adaptation is a well-documented polygenic trait and it can operate through different combinations of loci. Differentiated frequencies of "cold-adapted" variants favoured at lower temperatures and "warm-adapted" variants at higher temperatures reflect adaptation over a relatively short timescale and show that genetic variation that evolved in the native habitat is a primary factor allowing rapid adaptation across a thermal gradient in a new habitat (Barghi *et al.*, 2019; Krehenwinkel, Rödder & Tautz, 2015; Popovic & Riginos, 2020; Yang *et al.*, 2022). This suggests that invasion bottlenecks have a limited capacity to attenuate polygenic traits. The genetic redundancy resulting from polygenic adaptation may be an important mechanism facilitating adaptation in invasive species (Láruson, Yeaman & Lotterhos, 2020). Among population studies ($N = 101$), we identified 62% that included

both native and invasive populations, 35% that studied only invasive populations, while in three studies only native populations were analysed. Only 15% of population studies focused on selection footprints in both native and invasive populations (Fig. 1C), where mechanisms in native populations were investigated for their role in facilitating adaptation in invasive populations. Examples of mechanisms responsible for creating adaptive genomic diversity in native habitats that contributed to the diversity increase upon introduction in the invasive ranges are listed in Table 2.

Although the importance of adaptation in the native habitat has been acknowledged by a number of studies, the exact

Table 2. Examples of different selection mechanisms acting in the native range that may contribute to diversity increase upon invasion.

		Examples	References
Mechanisms promoting maintenance of high levels of diversity	Balancing selection	*Balancing selection in the native habitat that is driven by fluctuating environmental conditions and followed by directional selection in the invasive range.* In the copepod *Eurytemora affinis*, salinity fluctuations in the native environment and short, overlapping generations foster balancing selection (Stern & Lee, 2020). Directional selection occurred in the invasive populations at ion transport genes, which were under balancing selection in the native habitat. *Balancing selection in both the native and invasive range, maintaining multiple variants of specific genes.* In Eastern mosquitofish, *Gambusia holbrooki* (Vera *et al.*, 2016) genes related to reproduction, growth and development were under balancing selection in both the native and invasive range despite a clear reduction in genetic diversity. In common raccoon (Konopiński *et al.*, 2023) single nucleotide polymorphisms (SNPs) located in several immune genes were maintained under balancing selection in native and invasive range.	Stern & Lee (2020); Konopiński *et al.* (2023); Vera *et al.* (2016); Mittan-Moreau *et al.* (2022)
	Sharing SGV (standing genetic variation) and gene reuse	*Rapid and repeatable phenotypic and genomic adaptation in the native and invasive habitats* of common ragweed *Ambrosia artemisiifolia*. Candidate adaptation loci overlapped between ranges. The consistency in habitat characteristics between native and invasive ranges suggests that shared standing genetic variation could increase the probability of gene reuse during rapid local adaptation (van Boheemen & Hodgins, 2020).	van Boheemen & Hodgins (2020)
	Polygenicity	*Polygenic adaptation to temperature* in potato ground beetle (Yang *et al.*, 2022) and European green crab (Tepolt & Palumbi, 2020). High temperature tolerance in European green crab (*Carcinus maenas*) can explain its worldwide invasiveness. Differentiated frequencies of "cold-adapted" and "warm-adapted" variants in different temperatures reflect adaptation on a short timescale and show that genetic variation that evolved in native habitat is a primary factor allowing rapid adaptation across a thermal gradient in its new habitat.	Yang *et al.* (2022); Tepolt & Palumbi (2020)
Mechanisms promoting specific variants: diversifying selection		*Differential diversifying selection acting at the same loci in native and invasive ranges.* Found in genes putatively related to boldness and anxiety behaviours, reproduction, and food intake suppression in the red lionfish (*Pterois volitans*) (Burford Reiskind *et al.*, 2019). In Asian tiger mosquito (*Aedes albopictus*) reported in genes related to lipid metabolism and juvenile hormone-binding protein (Goubert *et al.*, 2017). *Diversifying selection in the native range followed by directional selection in the invasive range.* In house crow (*Corvus splendens*) reported in genes related to environmental adaptation.	Burford Reiskind *et al.* (2019); Lin *et al.* (2017); Konopiński *et al.* (2023); Goubert *et al.* (2017); Tepolt & Palumbi (2020); Krzemińska *et al.* (2018)
Unspecified mechanism		*Allele frequency change between native and introduced populations.* Studies do not specify the selection footprint, only report specific outlier loci in both ranges.	Merel *et al.* (2021); Narum *et al.* (2017); Flanagan *et al.* (2021); Helliwell *et al.* (2018)

causes of its presence are sometimes difficult to identify. For example, within the native and invasive ranges of the house crow (*Corvus splendens*), signatures of natural selection were detected in the mitogenome, but these were associated both with lineage diversification and recent adaptation to environmental conditions (Krzemińska *et al.*, 2018). In the invasive Asian tiger mosquito (*Aedes albopictus*), adaptive genetic and morphometric variation observed along the climatic gradient of the native range suggests that colonisation of northern latitudes promoted adaptation to cold environments prior to its worldwide invasion (Sherpa, Blum & Després, 2019b). Another genomic study on *A. albopictus* invasion (Goubert *et al.*, 2017) found selection footprints both in native and invasive populations, but it was impossible to delineate whether these were related to adaptations prior to invasion or were a recent shift in allele frequencies that reflects adaptations to cooler invasive habitats.

Adaptations that evolve in the native range are often affected by genetic drift upon establishment of invasive populations. A joint effect of demographic and selective forces affecting levels of genetic diversity and allele frequency change was observed in 9 out of 14 articles studying invasive and native populations where both selection and genomic drift were identified. In all but one of those cases, loss of genetic diversity was counteracted by gene flow between differentiated source populations in the invasive range. For example, in Chinook salmon (*Oncorhynchus tshawytscha*) (Narum *et al.*, 2017), exposure to novel pathogens in native populations resulted in selection on immune-related single nucleotide polymorphisms (SNPs) but the observed genomic pattern was then erased by genetic drift and gene exchange between different source populations in the introduced environments. Despite clear evidence of the presence of adaptive alleles in the native range and transmission of this genetic diversity into the invasive range, associations that should arise in the invasive range are sometimes not observed, suggesting the loss of adaptive capacity. For example, populations of sweet vernal grass (*Anthoxanthum odoratum*) were adapted to soil aluminium toxicity in their native range, but not invasive range, despite the presence of specific allelic variants (Gould & Gerber, 2016). This lack of association could be explained by genetic linkage or genetic background effects that may be different in the native and introduced ranges.

Findings from the studies linking pre-introduction adaptation in the native range with increased invasion ability illustrate the significance of prior adaptation for invasion success in new habitats. However, the number of such studies is limited and there are several reasons why we lack evidence. First, to detect such associations, the identification of source populations of an invasion is necessary. Further, both native and invasive populations must be sampled and sequenced. Precise demographic inferences, such as population substructure in both ranges, changes in effective population sizes, and levels of gene flow in both native and invasive populations also need to be estimated (Teshima, Coop & Przeworski, 2006). Additionally, the appropriate statistical measures that take into account demographic events must be applied,

especially in the case of balancing selection. When detecting multiple variants at intermediate frequencies at the population level, the results can be confounded by demographic processes. Moreover, the effects of selection, both in native and invasive populations, ideally should be experimentally tested or associated with environmental variables to demonstrate adaptation.

(2) Adaptation in the invasive range

Once a species has invaded, adaptation in the invasive habitat enhances its ability to persist and thrive by rapidly shifting its ecological niche to match the new environment better (Sultan *et al.*, 2013; Vandepitte *et al.*, 2014). Rapid acclimation to the new ecological niche can be realised by phenotypic plasticity (Promy, Newberry & Gulisija, 2023; Uller & Leimu, 2011) or direct genetic changes altering functional diversity resulting from natural selection. Here, we focus on genomic mechanisms enabling adaptation. We analyse sources of variation and processes creating genomic diversity, different selection footprints, and signatures of recent selection.

(a) Sources of genetic diversity: standing genetic variation, de novo mutations and presence of selective sweeps

Evolution requires genetic diversity (Clarke, 1979). Selection in the invasive range may act on new mutations or on standing genetic variation (Barrett & Schluter, 2008), leaving a different pattern in the genome. A novel, large-effect mutation that arises on a single haplotype in a population and ultimately reaches fixation creates a pattern of hard selective sweep. Soft sweeps generally refer to scenarios in which multiple haplotypes carry a beneficial variant that was present in the native range as standing genetic variation (Hernández *et al.*, 2011). To date, mounting evidence indicates that although hard selective sweeps are easier to detect in the genome than soft selective sweeps, they might not be the dominant mode of adaptation in many species. Further, identifying the relative contribution of standing *versus de novo* variation to rapid adaptation of invasive species is difficult, as high genomic marker density and reference genome contiguity are required for the identification of *de novo* variation. The degree to which beneficial *de novo* mutations can impact invasion success over the short timescale of invasion is also unclear (Pélissié *et al.*, 2018). In our search we did not locate any study that clearly confirmed the presence of adaptive *de novo* genetic diversity that arose in the invasive range and allowed adaptation there. In four studies we found a suggestion that adaptive changes can have a *de novo* origin (Kotsakiozi *et al.*, 2017; Lin *et al.*, 2017; Mérel *et al.*, 2021; Sherpa *et al.*, 2019c), but none of these studies performed a formal test proving the origin of this diversity. Standing genetic variation as a source of adaptive variants was reported in 24 out of 101 studies, the remaining studies did not specify the source of variation. In nine studies, homozygosity runs or regions with linkage disequilibrium (LD) were

interpreted as a footprint of selective sweeps (Wu *et al.*, 2019; Lin *et al.*, 2017; Krehenwinkel *et al.*, 2015; Chen *et al.*, 2021b; Hübner *et al.*, 2022; Liu *et al.*, 2022; Wegner, Lokmer & John, 2020; Konorov *et al.*, 2021; Yoshida *et al.*, 2016), but without a clear distinction between soft or hard sweep. It is important to note that the length of the selective sweep footprint left on genomic regions depends strongly on recombination rate (Garud, 2023) which can greatly narrow down the hitchhiking effect (Fay & Wu, 2000).

(b) Selection footprints in the invasive range

Adaptation of species in the invasive range results from different types of selection, including directional, divergent, or parallel selection. In cases where multiple genetic variants provide better adaptation to new environments, balancing selection occurs in the invasive range. Conversely, if variants brought into a new range confer lower fitness in the novel conditions, negative or purifying selection is expected.

The most common result reported as a proof of selection in the invasive range was a change in allele frequency (outlier loci) between native and introduced populations (7 out of 36 studies). These studies neither interpreted further the type of selection nor related outliers to specific selective pressures. Our survey did not find evidence that balancing or diversifying selection occurring in the native range (Lee, 2016) is the common driver of directional selection in the invasive range. Nevertheless it is important to note that although balancing selection is increasingly acknowledged as a widespread mechanism creating genetic diversity within populations, its detection is challenging. For genomic data, high SNP densities are needed as signatures of balancing selection are often much narrower than for other selection types (Bitarello *et al.*, 2023). The most prominent examples of the role of balancing selection in the native range for the success of invasive species show a repetitive response to selection acting on the same loci in native and invasive populations [*Eurytemora affinis* (Stern & Lee, 2020), *Gambusia holbrooki* (Vera *et al.*, 2016)]. Similarly, in the invasive European green crab (*Carcinus maenas*), adaptation to wide temperature spectra enabled successful adaptation in the invasive range (Tepolt & Palumbi, 2020). Five studies reported balancing selection in the invasive range, and 12 out of 101 studies reported only the presence of outlier loci between native and invasive populations, without interpreting the type of selection. Our results show that favourable genetic variants brought from native ranges are often a source of rapid adaptation to novel habitat characteristics.

The genomic pattern left by selection in an invasive population linked to environmental selection agents can provide confirmation of rapidly occurring adaptations (Gautier, 2015). However, statistical associations between specific gene variants and environmental characteristics are relatively rare in our database. In only 12 out of 60 studies were such associations reported, offering direct evidence for adaptation to environmental variables in the new range. Gene–environment analyses (GEAs) require gathering environmental

data and sampling a relevant number of individuals. Additionally, large, repetitive genomes, and high levels of gene flow between diverse populations affecting population structure and causing a decrease in locally adaptive variants *via* genetic swamping, make GEAs challenging. The small number of such studies reveals a significant gap in research on adaptation in invasive species and indicates that defining relevant environmental characteristics or traits relevant for invasion success remains challenging. More examples documenting such associations will improve our understanding of the genetic architecture of local adaptation.

Regardless of the type of selection footprint detected in invasive populations, the majority of studies report the joint action of selection and bottleneck/genetic drift and high or stable levels of genetic diversity (Fig. 5A).

(c) Factors creating genomic diversity in populations showing adaptive changes in the invasive range

High standing genomic diversity is expected to be the primary cause of adaptive potential in invasive species (Tepolt, 2015), both for terrestrial and aquatic environments. We therefore evaluated the levels of genomic diversity in studies where adaptive selection was detected in the invasive populations (60% of 101 population studies, Fig. 5A). Almost half of the studies that detected selection in the invasive range did not show a reduction in genetic diversity. An increase (e.g. Errbii *et al.*, 2021; Bieker *et al.*, 2022; Wellband *et al.*, 2018), no change (e.g. Yoshida *et al.*, 2016; Popovic *et al.*, 2021; Ni *et al.*, 2018), or only a moderate decrease in genetic diversity in the invasive range (e.g. Hübner *et al.*, 2022; Yin *et al.*, 2021) was reported in 26 out of the 60 studies. For moderate reductions of diversity, the authors often argued that the observed reduction did not affect the species' ability to undergo rapid adaptive evolutionary change. Only 11 out of 60 studies reported a decrease in genomic diversity of invasive populations (Fig. 1G). Using the categorisation of genetic diversity in the invasive range, only 11 of the 60 studies reported low diversity (Fig. 1G) and associated it with genetic bottlenecks upon introduction.

Most studies 39/60 report genetic drift and/or bottleneck as a force shaping genetic diversity in invasive populations (see Section IV.2). In some cases, reduced genetic diversity of invasive populations was reinforced by both genetic drift/bottleneck and diversifying, directional or purifying selection (Wu *et al.*, 2019; Pérez-Portela *et al.*, 2018; Vera *et al.*, 2016; Sherpa *et al.*, 2018; Skrede *et al.*, 2021; Selechnik *et al.*, 2019; Tepolt *et al.*, 2022; Mittan-Moreau *et al.*, 2022; Chen *et al.*, 2021c). In the invasive brown rat, *Rattus norvegicus* in China (Chen *et al.*, 2021c), a decrease in genetic diversity was caused both by genetic drift and directional selection in loci related to lipid metabolism and immunity in the invasive range. The authors underlined the role of bottlenecks and genetic drift in facilitating purifying selection resulting in more effective elimination of deleterious alleles due to nonrandom mating in small populations. In the invasive European green crab, *Carcinus maenas*, successful invasion
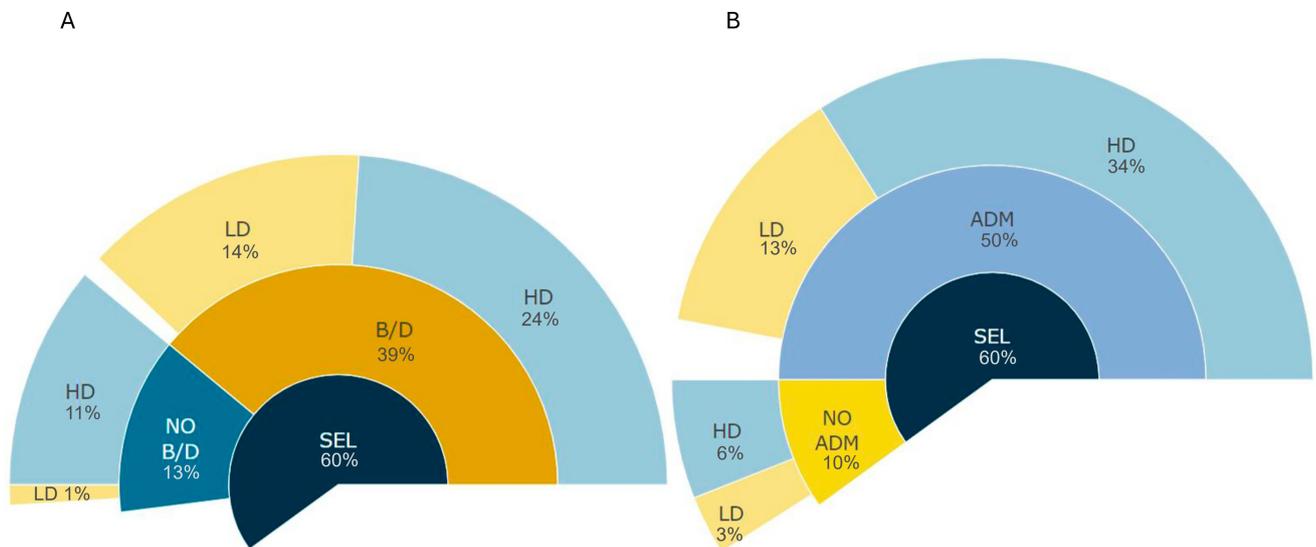
Fig. 5. Sunburst charts displaying the frequency of demographic factors affecting genomic diversity in articles where selection was detected: (A) the percentage of articles where selection was detected, the percentage of articles detecting the presence of genetic bottleneck and/or genetic drift (both or one of the mechanisms was assessed in the article) or absence (none of the mechanisms was confirmed in the article), and the percentage of articles where genetic diversity of invasive populations was assessed by the authors of each article as high or low; and (B) the percentage of articles where selection was detected, the percentage of articles where admixture was reported or not and the percentage of articles where genetic diversity of invasive populations was assessed as high or low. Empty regions reflect the lack of information in the analysed articles. ADM, presence of admixture; B/D, presence of genetic bottleneck and/or genetic drift; HD, high level of genetic diversity in invasive populations; LD, low level of genetic diversity in invasive populations; NO ADM, absence of admixture; NO B/D, absence of genetic bottleneck and genetic drift; SEL, presence of selection in invasive range.

along the northeast Pacific coastline occurred despite low levels of genetic diversity (Tepolt *et al.*, 2022). In this case, rapid cold adaptation acted through a handful of linked SNPs under balancing selection. The authors showed that variation originated from a single inversion polymorphism before the introduction into the invasive range. In fall webworm, *Hyphantria cunea*, selective sweeps and excess of non-synonymous mutations in gustatory receptors as well as expansion in gustatory receptor genes were found to be associated with novel nutrition sources despite strong bottleneck (Wu *et al.*, 2019). These examples show that while high genetic diversity can foster invasive potential, it is not a prerequisite for successful invasion.

The most common phenomenon contributing to an increase in levels of genomic diversity of invasive populations under selection was gene flow within the invasive range, with a majority (36/60) of studies identifying admixture between invasive populations as a primary factor increasing the genomic diversity of invasive populations (Section IV.3). Only 10 out of 60 studies reported no admixture, and 14 out of 60 reported a lack of significant change in genomic diversity. Other factors included recurrent invasions and the presence of bridgehead populations (Kramer *et al.*, 2023; Sherpa *et al.*, 2018) that eventually led to increased genetic variation. For example, the Caribbean invasive populations of the mosquito *Aedes aegypti* (Sherpa *et al.*, 2018) showed signs of genetic drift, two bottlenecks and small effective population size. As a result, invasive populations had overall low genetic diversity,

but recurrent invasion events eventually resulted in an increase of genetic diversity and potentially allowed for selection at loci related to metabolic breakdown of insecticides.

High levels of genetic diversity are also generated by introgression, either between divergent lineages of invasive species (Errbii *et al.*, 2021; Bieker *et al.*, 2022), from related native species (Yoshida *et al.*, 2016) or between related non-native species that occurred prior to introduction (Martin *et al.*, 2016). Only 16 out of 101 studies investigated interspecies hybridisation (Fig. 1D). Adaptive introgression was detected in seven studies, where introgression facilitated adaptation by introducing specific genes/variants into invasive species' genomes. Dutch elm disease is caused by three different fungal lineages *Ophiostoma ulmi*, *Ophiostoma novo-ulmi* subspecies *novo-ulmi* and *Ophiostoma novo-ulmi* subspecies *americana*, which differ in pathogenicity and temperature optimum. Gene flow from the more ancient to the more recent invasive lineages resulted in introgression between lineages that impacted fitness-related traits (Hessenauer *et al.*, 2020). Another example is provided by introgression between two species of *Helicoverpa* moths, where widespread introgression from native to invasive species took place, followed by intensive expansion of the invasive *H. armigera*. Interestingly, selection against introgression after the initial expansion, and back-introgression of an insecticide-resistant locus to native species, has resulted in the coexistence of two insecticide-resistant pest species (Valencia-Montoya *et al.*, 2020).

Taken together, the latest genomic studies on rapid adaptation during invasions provide evidence that while genetic drift often occurs as the result of the establishment of new populations outside their natural ranges, any reduction in genetic diversity typically is not severe and often is counteracted by multiple introductions, admixture or interspecific gene flow. A significant and permanent reduction of genetic diversity is rarely detected in invasive populations, and even where it occurs, does not appear to rule out the chances of rapid molecular adaptation and usually does not impact the species' performance in the invasive range. The classical "genetic paradox" of invasive species, where adaptation in the invasive range is observed despite reduction in genetic diversity, seems to be exceptionally rare.

Similar adaptive mechanisms operate in both the native and invasive populations, yet the latter may experience more pronounced or relaxed selection pressures depending on conditions in the invasive range. Heightened selection pressure from novel environmental conditions in the invasive range may impose selection for higher mutation rates (Travis & Travis, 2002). By contrast, release from natural enemies may reduce or eliminate selective forces in invasive populations (Biedrzycka et al., 2023). Additionally, the presence of other species or genetically distinct ecotypes of the invasive species may facilitate hybridisation and introgression in the invasive range. It can be argued that, in many cases, increased selection pressures in the invasive habitat could lead to accelerated evolutionary processes compared to the native habitat.

Studies using genomic data to explore molecular adaptation in invasive populations are undergoing a transition from analyses mainly of demographic processes to research aiming to understand the molecular mechanisms driving rapid adaptation in invasive species.

(d) Genes under selection

The superior performance of invasive species should imply that they possess adaptations in genes related to those specific biological features. Gene families known to be associated with invasiveness include families involved in chemosensory abilities (Liu et al., 2016; Papanicolaou et al., 2016; Sparks & Dickens, 2017; Wu et al., 2016; Yuan et al., 2016), detoxification metabolism (Andersen et al., 2017; Grigoraki et al., 2017; Nguyen et al., 2016; Tian et al., 2017), heat shock proteins (HSPs) (Hu, Chen & Li, 2014; Wang et al., 2017) and innate immunity (Beckert et al., 2016; Vogel, Schmidtberg & Vilcinskas, 2017). Selection processes were commonly detected in the invasive range, and 46 of those 60 articles identified specific genes or DNA regions acting as selection targets (Table 3). However, the majority of studies used only outlier detection and seldom linked outlier loci with specific selection pressures or assigned significance using quantile thresholds, thus some of the reported results might be false positives. Additionally, the inference between sectional and demographic processes should be taken into account when identifying sites under selection (Lotterhos & Whitlock, 2014). Several articles emphasise that finer resolution genotyping (Hübner et al., 2022), whole-genome sequencing, appropriately large sample sizes

(Jeffery et al., 2017a; van Boheemen & Hodgins, 2020), and the availability of reference genomes for invasive species (Mérel et al., 2021; Formenti et al., 2022; Theissinger et al., 2023) are needed for the identification of genes crucial for adaptation in the invasive range. We reiterate these recommendations here. For the studies in our database, genes under selection are related to various environmental pressures and functions (Table 3). Additional studies in different invasive systems are needed to identify other invasion-related gene families, and should include comparative genomic studies on gene family expansions and contractions performed on related invasive and non-invasive species.

New environmental conditions pose significant challenges for invasive species, particularly during establishment in a new range. Specific genes under environmental selection were identified in 19 articles where selection was detected (Table 3). Genetic adaptations to temperature were found in 10 of all analysed environmental-related studies and were key in populations invading colder climates, higher altitudes and/or facing seasonal variability (e.g. overwintering). Genes associated with lipid metabolic pathways constituted a significant subset of temperature-related genes. Lipid metabolic regulation is involved in adaptation to fluctuating temperatures, overwintering and diapause regulation (Table 3).

According to the enemy release hypothesis, pathogens left behind during species translocation should relax selection on the host genome (Colautti et al., 2004). However, genes associated with the immune response were found to be under selection in seven studies. Candidate genes often were linked to virus and helminth infection or were inflammatory response-related genes (Table 3). A response of immune-related genes to environmental stressors was detected in the marine ascidian *Molgula manhattensis* (Chen et al., 2021a).

Genes involved in metabolic pathways were under selection in nine articles. Most genes were linked to carbohydrate metabolism associated with nutrient processing (Table 3). Selection of genes linked to detoxification was found in several studies (Table 3).

Insecticide resistance represents a concern for pest or vector control programs (Kramer et al., 2023). A signature of selection in genes linked to insecticide resistance was reported in four studies (Table 3). Additionally, selection acting on olfactory and gustatory receptors was reported in several studies. Selection of genes associated with abiotic stressors was also reported in multiple studies.

VI. CONTRIBUTIONS OF DIFFERENT GENOMIC CHANGES IN INVASIVE SPECIES

Several studies in our database mention genetic changes other than sequence variation that could potentially impact the success of invasive populations, including genome size, whole genome duplication (WGD), gene family expansions, gene regulation, or activity of transposable elements (TE).

Table 3. Summary of functional categories of genes reported to be under selection in the studies included in our database.

Genes	Organisms	Function	References
Environmental pressure	*Eurytemora affinis*, marine ascidian, sea squirts	Adaptation to salinity	Chen *et al.* (2021a); Stern & Lee (2020); Lin *et al.* (2017); Ni *et al.* (2018)
	Oculina patagonica, *Mytilus galloprovincialis*, round goby, European green crab, wasp spider, Colorado potato beetle, yellow fever mosquito, Asian tiger mosquito, cane toad, house crow, sea squirts, brown rat, ant, Asian house rat	Adaptation to temperature	Lin *et al.* (2017); Sherpa *et al.* (2019b); Krehenwinkel *et al.* (2015); Goubert *et al.* (2017); Leydet *et al.* (2018); Popovic & Riginos (2020); Errbii *et al.* (2021); Chen *et al.* (2021b); Yang *et al.* (2022); Kramer *et al.* (2023); Tepolt & Palumbi (2020); Konorov *et al.* (2021); Krzemińska *et al.* (2018); Selechnik *et al.* (2019); Sherpa *et al.* (2019c); Wellband *et al.* (2018); Tepolt *et al.* (2022); Chen *et al.* (2021c)
	Asian tiger mosquito, ant, Asian house rat, house crow, brown rat	Lipid metabolic regulation	Sherpa *et al.* (2019b); Goubert *et al.* (2017); Errbii *et al.* (2021); Chen *et al.* (2021b); Konorov *et al.* (2021); Krzemińska *et al.* (2018); Chen *et al.* (2021c)
	Asian house rat	Adaptation to high-altitude hypoxia	Chen *et al.* (2021c)
Metabolism	Mealybug, tubenose goby, sweet vernal grass, common ragweed	Detoxification	Ma *et al.* (2020); Bieker *et al.* (2022); Wellband *et al.* (2018); Gould & Geber (2016)
	Pacific oyster, *Eurytemora affinis*, fall webworm, mealybug, sea lamprey	Energy metabolism	Wu *et al.* (2019); Stern & Lee (2020); Ma *et al.* (2020); Wegner *et al.* (2020); Yin *et al.* (2021)
Growth, reproduction, development	Red lionfish, common ragweed, small hive beetle	Reproduction	Burford Reiskind *et al.* (2019); Bieker *et al.* (2022); Liu *et al.* (2021)
	Common ragweed, small hive beetle, mealybug, sea lamprey, mosquitofish, guppies, marine mussel, threespine stickleback, Florida Burmese pythons	Growth and development	Ma *et al.* (2020); Rosenthal *et al.* (2021); Vera *et al.* (2016); Card *et al.* (2018); Popovic & Riginos (2020); Bieker *et al.* (2022); Liu *et al.* (2021); Yin *et al.* (2021), Yoshida *et al.* (2016); Martin *et al.* (2016)
Insecticide resistance	*Helicoverpa* moths, Colorado potato beetle, *Halyomorpha halys*, yellow fever mosquito	Insecticide resistance	Yang *et al.* (2022); Parvizi *et al.* (2023); Kramer *et al.* (2023); Sherpa *et al.* (2018); Valencia-Montoya *et al.* (2020); Parvizi *et al.* (2023)
Immune	Common raccoon, Australian rabbits, *Eurytemora affinis*, Pacific oyster, threespine stickleback, common ragweed, marine ascidian	Immune response	Chen *et al.* (2021a); Stern & Lee (2020); Konopiński *et al.* (2023); Hodgins *et al.* (2015); Bieker *et al.* (2022); Wegner *et al.* (2020); Yoshida *et al.* (2016); Martin *et al.* (2016); Schwensow *et al.* (2017)
Others	Fall webworms, *Drosophila suzukii*, ants, *Halyomorpha halys*	Olfactory and gustatory functions	Koch *et al.* (2020); Wu *et al.* (2019); Errbii *et al.* (2021); Parvizi *et al.* (2023)
	Dry rot fungus	DNA replication and protein modification	Skrede *et al.* (2021)
	Common ragweed, marine ascidian, round goby, marine mussels, red turpentine beetle, glossy buckthorn, *Mikania micrantha*, *Helianthus annus*, *Ambrosia trifida*, *Ambrosia artemisiifolia*, *Centaurea diffusa*, *Centaurea solstitialis*, *Cirsium arvense*	Response to abiotic stressors	Chen *et al.* (2021a); Hodgins *et al.* (2015); Popovic & Riginos (2020); Bieker *et al.* (2022); Liu *et al.* (2022); De Kort *et al.* (2016); Yang *et al.* (2017); Wellband *et al.* (2018)

Studies of intraspecific genome size variation in plants suggest that a smaller amount of nuclear DNA may be a factor in successful invasions in resource-poor environments; invasive populations were characterised by a smaller genome in European common reed grass in North America (see Fig. 2 in Pyšek *et al.*, 2018), and a smaller genome size was also an

important explanatory factor for differences in functional plant traits for successful invasions (see Fig. 3 in Pyšek *et al.*, 2018). By contrast, allopolyploids that have evolved as a result of merging the subgenomes of different species offer evolutionary novelty through biased homoeologous gene expression and TE repression possibly enhancing adaptiveness and invasiveness associated with the species' traits and ecology (Giraud *et al.*, 2021). WGD events are also a common cause of the development of increased stress resistance in plants due to advantageous changes in gene family sizes, and have contributed to invasiveness in notorious weeds (Qian *et al.*, 2022). Paleoploidic history in combination with lineage-specific and recent segmental genomic duplications can lead to rapid adaptation to new habitats, especially when gene families related to fast growth are significantly expanded (Liu *et al.*, 2020b). Another key adaptation is the capacity of invasive species to establish large clonal populations, as has been shown in parthenogenically reproducing species (Gutekunst *et al.*, 2018). Their evolutionary success lies primarily in the potential to use increased heterozygosity associated with polyploidy and clonal expansion for rapidly acquired adaptive advantages (Gutekunst *et al.*, 2018).

Some studies used experimental approaches to demonstrate the importance of particular invasive traits, such as the upregulation of key genes, and provided additional evidence for mechanisms facilitating invasion (Wu *et al.*, 2019; Rispe *et al.*, 2020; Liu *et al.*, 2020a; Liu *et al.*, 2018; Zhang *et al.*, 2020; Giraud *et al.*, 2021). Studies comparing gene expression patterns between native and invasive populations under stress showed that expression responses to stress changes, indicating genetic differentiation of invasive populations, involves the rewiring of regulatory networks (Marín *et al.*, 2021; Oh *et al.*, 2022).

TEs, in general, generate a cost in the host genome and are usually removed or inactivated. Founder events can create periods during which the strength of purifying selection is weaker, facilitating activation and expansion of such elements, as has been demonstrated in a fungal pathogen (Oggenfuss *et al.*, 2021). This can shape and diversify the genomes of invasive species. Only two studies in our database analysed TEs in the context of invasion, providing evidence for the association of some active TE families with invasive populations (Mérel *et al.*, 2021; Lee & Wang, 2018). Still, the impact of TEs on fitness and adaptation or maladaptation in invasive populations remains to be studied further.

Genetic factors contributing to invasion success extend beyond SNPs and often require well-annotated genomes and experimental testing. However, they offer a broader perspective on mechanisms of adaptation and in particular on adaptation of invasive species to new environments.

VII. NON-GENETIC FACTORS CONTRIBUTING TO INVASION

Our search terms were related to mechanisms that could potentially affect the genetic diversity and performance of invasive species. The collected articles also were screened for information on non-genetic mechanisms that could explain the invasion success of particular species. Only 13 of all analysed articles mentioned non-genetic factors as contributing to successful invasion. By qualifying the mechanism as non-genetic, we excluded information that the invasion was human-mediated, as invasions are inherently related to human activity. We also excluded factors that may have a genetic background, but where their elucidation was not the purpose of the study (i.e. factors that were mentioned in the text but not directly studied such as the breath of thermal tolerance or extreme insecticide resistance).

The most frequently mentioned non-genetic factor was habitat niche congruence between native and invasive ranges (Green *et al.*, 2023; Du *et al.*, 2021; Sherpa *et al.*, 2019c; Gutekunst *et al.*, 2018), although only one of these studies performed niche comparison analysis (Sherpa *et al.*, 2019c). Niche comparisons of Asian tiger mosquito invasion in Europe supported niche conservatism, suggesting that the invasive range expansion did not necessitate new genomic adaptations, although genomic adaptations were detected in invasive populations. Other mechanisms included "enemy release" in the invasive range (Bieker *et al.*, 2022) and the composition of the gut microbiome (Liu *et al.*, 2018).

The infrequent references to non-genetic factors promoting invasions suggest that authors often concentrate on individual factors, and that multidisciplinary studies are rarely undertaken. Although this approach is understandable, given the time and cost demands of comprehensive studies, it complicates an holistic understanding of the invasion process. Conversely, it may suggest that mechanisms related to genomic diversity may predominate.

VIII. DISCUSSION AND FUTURE DIRECTIONS

There is an inherent bias related to studying successful species invasions that affects our understanding of invasion mechanisms. Although it is difficult to study failed invasions, research into the causes of failed biocontrol and studies of historical data (Marsico *et al.*, 2010) can provide a context in which to assess successful invasions. Such data sets are limited, but the awareness of the costs of alien species introduction is growing. According to the EU Invasive Alien Species Regulation (1143/2014), strict monitoring of all stages of species invasions must be undertaken and there is a requirement for science-based decision making. These regulations should encourage scientists to study early stages of invasions and identify causes of varying invasion success. Below we describe the general picture emerging from the latest invasion genomics studies, present a critical assessment of gaps in the field and propose directions for future research.

The frequent role of admixture in elevating the levels of genetic diversity in novel invasive habitats is the most important finding of our study. Although admixture can be detected using data from well-adopted and affordable techniques, such as RRS and standard population genetics tests,

we argue that there should be scope for other approaches to answer more detailed questions and understand the consequences of admixture. Precise identification of the time and extent of admixture between divergent populations in the invasive range, categorisation of invasive populations according to the degree of admixture, and identification of "admixture hotspots" should be a focus in invasive species management. Moreover, studies measuring fitness change or an increase/decrease in invasiveness after admixture will be necessary to assess its role in adaptation of invasive species. We propose the term "Invasive Evolutionarily Significant Unit" (IESU) to be used in management of invasive populations. This term extends the longstanding concept of Evolutionarily Significant Unit (ESU) in conservation genetics. An ESU represents a population or a group of populations evolving independently and managed for effective species conservation (Hoelzel, 2023). We recommend designating invasive population groups or spatial areas to IESUs where a high level of admixture has possibly increased the evolutionary potential of an invasive species. RRS approaches should be adopted as an inexpensive and widely accessible tool for the identification of admixture hotspots to which proactive management actions should be directed.

We also see the need to collect and analyse time-point series data which are crucial for detecting selection and demographic events at different stages of invasion. Time-series sampling would allow precise detection and estimation of the frequency and timing of admixture events and bottlenecks occurring upon introduction in the new range and the tempo of evolutionary change. Additionally, they could help determine if selection occurred immediately after introduction, whether it required a lag phase, or took place during population expansion. Although we identified collected numerous studies that compared the genetic diversity of native and invasive populations, most of these were point measurements that do not allow us to evaluate precisely when a change in genomic diversity occurred. Collecting time-series data for invasive species will be challenging as many invasions are only identified at later stages. Nevertheless, increasing awareness of the risk potentially posed by alien species, especially from rapidly spreading and fast-reproducing groups, means that genetic monitoring of such taxa could enable the collection of these vital data.

Our review found the most common evidence of adaptation was the detection of outlier loci between native and invasive ranges. Outlier loci can be identified with a variety of genomic methods, with different selection criteria, and with or without taking into account the underlying demographic history. Such methods do not inform us about the extent of a species' response to selection but rather identify potential genetic targets of selection. In the absence of information on environmental features responsible for the change, or experimental manipulation of outlier loci, this approach does not allow biological interpretation of the selection process. Analysing selection in both invasive and native ranges, for example using GEA analysis, with respect to habitat heterogeneity, instead provides a comprehensive understanding of the role of the adaptive process in invasions. Furthermore, measurements of the level of genetic diversity that is needed for a species to adapt to environmental change can be of use when modelling species responses to climate change. Data on adaptive potential expressed as variation in functional loci and the levels of genomic diversity should be incorporated into species distribution and ecological niche modelling. Studies of native and invasive populations inhabiting differentiated habitats should provide evidence for the extent of parallel evolution in invasive populations. Invasive species could serve as excellent models for measuring and validating genetic offset, that is a measure of the mismatch in the genotype–climate association between current and future climates (Fitzpatrick & Keller, 2015), and for evolutionary rescue in the face of climate change. Invasive populations represent natural experiments, and hence enable comparisons of population fitness in new environments with fitness expected from GEA predictions.

Incorporating fitness, population performance or phenotypic data into the study of adaptations in invasive species requires controlling for environmental variation, often by using experimental designs. Identification of the most common basis of phenotypic variation encoded by multiple small-effect loci can be achieved by controlling for environmental variation and using the genome wide association studies (GWAS) approach. GWAS allows for identification of species characteristics that are crucial for effective invasion such as dispersal, reproduction and resistance to control measures (Blackburn et al., 2024). We did not find evidence of *de novo* mutations being a frequent source of diversity and adaptation in invasive populations, despite previous assertions, not only for invasive species but for the process of adaptation in general (Capblancq et al., 2020). In our database, standing genetic variation appears to be the most commonly detected, but also most commonly investigated, source of adaptation in invasive populations. Our lack of detection of a role for *de novo* mutations could be the result of the still limited number of studies using high-coverage WGS data.

Although the cost of WGS technologies, especially long-read sequencing, is still prohibitive for many researchers, the number of reference genomes available is gradually increasing. Projects such as Earth BioGenome Project (EBP) or European Reference Genome Atlas (ERGA) are producing high-quality genomic sequences across eukaryotic diversity (Formenti et al., 2022). Numerous invasive species still lack reference genomes (Matheson & McGaughran, 2022), but we expect a rapidly increasing availability of WGS data. Importantly, even when WGS data were used in invasion studies, the full potential of the data often was not used to elucidate the mechanisms of invasions. Fine resolution of genetic diversity and population structure provided by WGS ensures accurate estimation of effective population size (Ne), recombination rate, linkage disequilibrium or mutation load. Nevertheless, many studies using sequenced genomes relied only on SNP data. WGS data allow us to analyse structural variation (SV) that should be considered when determining local adaptation. Large-effect genetic changes

may be achieved by groups of mutations in tight genetic linkage (Yeaman & Whitlock, 2011), including mutations captured by chromosomal inversions. There is growing evidence that inversions can drive range expansions (Kirkpatrick & Barrett, 2015). Comparisons of SV among invasive and non-invasive species could provide insights into the role of specific genome features in adaptation during invasion.

We also noted a lack of comparative genomic studies that attempted to identify genome characteristics other than SNPs underlying species invasiveness. In their new range, invasive species typically experience a new climate, availability of resources and biotic interactions. Adaptation to these new environmental conditions sometimes requires novel genetic variation that may provide new functions important for facing new environmental challenges. Such sudden shifts in selective pressures may result in large-effect mutations (Orr, 2005). The sources of novelty are often gene duplications, resulting in the expansion of gene families and subsequent neofunctionalisation of specific genes (Salojärvi, 2019). Comparison of gene family sizes between genomes of related native and invasive species and selection acting on these genes can provide insights into how genomic novelty enhances adaptive responses (Pyšek et al., 2018).

Genome-wide studies allow identification of genes and molecular mechanisms crucial for understanding the success of invasive species and for developing countermeasures (Heuertz et al., 2023). The application of genomic data to prevent or manage invasions has been restricted to certain groups of invasive organisms, such as in the biosurveillance of forest insect pests (Roe et al., 2019) or crop pests (Taylor et al., 2012; Wani et al., 2022). Examples of applications in other groups of invasive species are still limited (but see Ferreira-Martins et al., 2021; Harvey-Samuel, Ant & Alphey, 2017) and are currently under development. This application gap highlights the potential future directions of WGS, which should prioritise controlling invasive species to promote biodiversity conservation and safeguard ecosystem services.

IX. CONCLUSIONS

(1) This systematic review of articles using genomic methods to reveal mechanisms of species invasions shows that the "genetic paradox" of invasive species is not a common phenomenon.
(2) Demographic processes, such as genetic drift and bottlenecks rarely cause a significant decrease in genomic diversity. Any reduction in genomic diversity usually was described as relatively mild and almost always resolved *via* gene flow between different invasive populations.
(3) Even where a decrease in genomic diversity between native and invasive ranges was noted, the overall level of genomic diversity in invasive populations usually remained high. Selection processes could be detected in more than half

of the analysed studies, further demonstrating that reductions in diversity do not prevent adaptations to novel habitats.
(4) Despite a large number of studies involving genomics in invasion studies, there remains a need for wider application of time-point series genomic data, the incorporation of habitat, climate and population fitness components into genomic analysis, and more frequent application of WGS data both for revealing invasion mechanisms and for invasion management.

X. REFERENCES

References identified with an asterisk (*) are cited only within the online Supporting Information.

*ADRIAN-KALCHHAUSER, I., BLOMBERG, A., LARSSON, T., MUSILOVA, Z., PEART, C. R., PIPPEL, M., SOLBAKKEN, M. H., SUURVÄLI, J., WALSER, J.-C., WILSON, J. Y., ALM ROSENBLAD, M., BURGUERA, D., GUTNIK, S., MICHIELS, N., TÖPEL, M., ET AL. (2020). The round goby genome provides insights into mechanisms that may facilitate biological invasions. *BMC Biology* **18**(1), 11.

*ALVES, J. M., CARNEIRO, M., DAY, J. P., WELCH, J. J., DUCKWORTH, J. A., COX, T. E., LETNIC, M., STRIVE, T., FERRAND, N. & JIGGINS, F. M. (2022). A single introduction of wild rabbits triggered the biological invasion of Australia. *Proceedings of the National Academy of Sciences* **119**(35), e2122734119.

ANDERSEN, J. C., HAVILL, N. P., CACCONE, A. & ELKINTON, J. S. (2017). Postglacial recolonization shaped the genetic diversity of the winter moth (*Operophtera brumata*) in Europe. *Ecology and Evolution* **7**(10), 10.

*ARIAS, M. B., HARTLE-MOUGIOU, K., TABOADA, S., VOGLER, A. P., RIESGO, A. & ELFEKIH, S. (2022). Unveiling biogeographical patterns in the worldwide distributed *Ceratitis capitata* (medfly) using population genomics and microbiome composition. *Molecular Ecology* **31**(18), 4866–4883.

BAKER, H. G. & STEBBINS, G. L. (1965). The Genetics of Colonizing Species. In *Proceedings of the First International Union of Biological Sciences Symposia on General Biology*.

BARGHI, N., TOBLER, R., NOLTE, V., JAKŠIĆ, A. M., MALLARD, F., OTTE, K. A., DOLEZAL, M., TAUS, T., KOFLER, R. & SCHLÖTTERER, C. (2019). Genetic redundancy fuels polygenic adaptation in *Drosophila*. *PLoS Biology* **17**(2), 2.

*BARKER, B. S., ANDONIAN, K., SWOPE, S. M., LUSTER, D. G. & DLUGOSCH, K. M. (2017). Population genomic analyses reveal a history of range expansion and trait evolution across the native and invaded range of yellow starthistle (*Centaurea solstitialis*). *Molecular Ecology* **26**(4), 1131–1147.

BARRETT, R. D. H. & SCHLUTER, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution* **23**(1), 38.

BECKERT, A., WIESNER, J., SCHMIDTBERG, H., LEHMANN, R., BAUMANN, A., VOGEL, H. & VILCINSKAS, A. (2016). Expression and characterization of a recombinant i-type lysozyme from the harlequin ladybird beetle *Armonia axyridis*. *Insect Molecular Biology* **25**(3), 202–215.

BELLARD, C., CASSEY, P. & BLACKBURN, T. M. (2016). Alien species as a driver of recent extinctions. *Biology Letters* **12**(2), 20150623.

*BERGLAND, A. O., TOBLER, R., GONZÁLEZ, J., SCHMIDT, P. & PETROV, D. (2016). Secondary contact and local adaptation contribute to genome-wide patterns of clinal variation in *Drosophila melanogaster*. *Molecular Ecology* **25**(5), 1157–1174.

BIEDRZYCKA, A., KONOPIŃSKI, M. K., POPIOŁEK, M., ZAWIŚLAK, M., BARTOSZEWICZ, M. & KLOCH, A. (2023). Non-MHC immunity genes do not affect parasite load in European invasive populations of common raccoon. *Scientific Reports* **13**(1), 15696.

BIEKER, V. C., BATTLAY, P., PETERSEN, B., SUN, X., WILSON, J., BREALEY, J. C., BRETAGNOLLE, F., NURKOWSKI, K., LEE, C., BARREIRO, F. S., OWENS, G. L., LEE, J. Y., KELLNER, F. L., VAN BOHEEMEN, L., GOPALAKRISHNAN, S., ET AL. (2022). Uncovering the genomic basis of an extraordinary plant invasion. *Science Advances* **8**(34), eabo5115.

BITARELLO, B. D., BRANDT, D. Y. C., MEYER, D. & ANDRÉS, A. M. (2023). Inferring balancing selection from genome-scale data. *Genome Biology and Evolution* **15**(3), evad032.

BLACKBURN, G. S., KEELING, C. I., PRUNIER, J., KEENA, M. A., BÉLIVEAU, C., HAMELIN, R., HAVILL, N. P., HEBERT, F. O., LEVESQUE, R. C., CUSSON, M. & PORTH, I. (2024). Genetics of flight in spongy moths (*Lymantria dispar ssp.*): functionally integrated profiling of a complex invasive trait. *BMC Genomics* **25**(1), 541.

BOCK, D. G., CASEYS, C., COUSENS, R. D., HAHN, M. A., HEREDIA, S. M., HÜBNER, S., TURNER, K. G., WHITNEY, K. D. & RIESEBERG, L. H. (2015). What we still don't know about invasion genetics. *Molecular Ecology* **24**(9), 2277–2297.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E. & Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* **144**(1), 1.

*Brazier, T., Cherif, E., Martin, J.-F., Gilles, A., Blanchet, S., Zhao, Y., Combe, M., McCairns, R. J. S. & Gozlan, R. E. (2022). The influence of native populations' genetic history on the reconstruction of invasion routes: the case of a highly invasive aquatic species. *Biological Invasions* **24**(8), 2399–2420.

Brian, J. I. & Catford, J. A. (2023). A mechanistic framework of enemy release. *Ecology Letters* **26**(12), 2147–2166.

Burford Reiskind, M. O., Reed, E. M. X., Elias, A., Giacomini, J. J., McNear, A. F., Nieuwsma, J., Parker, G. A., Roberts, R. B., Rossi, R. E., Stephenson, C. N., Stevens, J. L. & Williams, B. E. (2019). The genomics of invasion: characterization of red lionfish (*Pterois volitans*) populations from the native and introduced ranges. *Biological Invasions* **21**(7), 2471–2483.

*Burrell, A. M., Pepper, A. E., Hodnett, G., Goolsby, J. A., Overholt, W. A., Racelis, A. E., Diaz, R. & Klein, P. E. (2015). Exploring origins, invasion history and genetic diversity of *Imperata cylindrica* (L.) P. Beauv. (Cogongrass) in the United States using genotyping by sequencing. *Molecular Ecology* **24**(9), 2177–2193.

*Cao, L.-J., Li, B.-Y., Chen, J.-C., Zhu, J.-Y., Hoffmann, A. A. & Wei, S.-J. (2021). Local climate adaptation and gene flow in the native range of two co-occurring fruit moths with contrasting invasiveness. *Molecular Ecology* **30**(17), 4204–4219.

Capblancq, T., Fitzpatrick, M. C., Bay, R. A., Exposito-Alonso, M. & Keller, S. R. (2020). Genomic prediction of (mal)adaptation across current and future climatic landscapes. *Annual Review of Ecology, Evolution, and Systematics* **51**(1), 245.

Card, D. C., Perry, B. W., Adams, R. H., Schield, D. R., Young, A. S., Andrew, A. L., Jezkova, T., Pasquesi, G. I. M., Hales, N. R., Walsh, M. R., Rochford, M. R., Mazzotti, F. J., Hart, K. M., Hunter, M. E. & Castoe, T. A. (2018). Novel ecological and climatic conditions drive rapid adaptation in invasive Florida Burmese pythons. *Molecular Ecology* **27**(23), 4744–4757.

Chapple, D. G., Miller, K. A., Kraus, F. & Thompson, M. B. (2013). Divergent introduction histories among invasive populations of the delicate skink (*Lampropholis delicata*): has the importance of genetic admixture in the success of biological invasions been overemphasized? *Diversity and Distributions* **19**(2), 134.

Charlesworth, D. & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics* **10**(11), 11.

*Chen, X.-G., Jiang, X., Gu, J., Xu, M., Wu, Y., Deng, Y., Zhang, C., Bonizzoni, M., Dermauw, W., Vontas, J., Armbruster, P., Huang, X., Yang, Y., Zhang, H., He, W., ET AL. (2015). Genome sequence of the Asian Tiger mosquito, *Aedes albopictus*, reveals insights into its biology, genetics, and evolution. *Proceedings of the National Academy of Sciences* **112**(44), E5907–E5915.

Chen, Y., Gao, Y., Huang, X., Li, S. & Zhan, A. (2021a). Local environment-driven adaptive evolution in a marine invasive ascidian (*Molgula manhattensis*). *Ecology and Evolution* **11**(9), 4252–4266.

Chen, Y., Hou, G., Jing, M., Teng, H., Liu, Q., Yang, X., Wang, Y., Qu, J., Shi, C., Lu, L., Zhang, J. & Zhang, Y. (2021b). Genomic analysis unveils mechanisms of northward invasion and signatures of plateau adaptation in the Asian house rat. *Molecular Ecology* **30**(24), 6596–6610.

Chen, Y., Zhao, L., Teng, H., Shi, C., Liu, Q., Zhang, J. & Zhang, Y. (2021c). Population genomics reveal rapid genetic differentiation in a recently invasive population of *Rattus norvegicus*. *Frontiers in Zoology* **18**(1), 6.

Clarke, B. C. (1979). The evolution of genetic diversity. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **205**(1161), 453.

Colautti, R. I., Ricciardi, A., Grigorovich, I. A. & MacIsaac, H. J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters* **7**(8), 721.

*Comeault, A. A., Wang, J., Tittes, S., Isbell, K., Ingley, S., Hurlbert, A. H. & Matute, D. R. (2020). Genetic diversity and thermal performance in invasive and native populations of African Fig Flies. *Molecular Biology and Evolution* **37**(7), 1893–1906.

Day, T. (2015). Information entropy as a measure of genetic diversity and evolvability in colonization. *Molecular Ecology* **24**(9), 2073.

De Kort, H., Mergeay, J., Jacquemyn, H. & Honnay, O. (2016). Transatlantic invasion routes and adaptive potential in North American populations of the invasive glossy buckthorn, Frangula alnus. *Annals of Botany* **118**(6), 1089–1099.

*Demené, A., Legrand, L., Gouzy, J., Debuchy, R., Saint-Jean, G., Fabreguettes, O. & Dutech, C. (2019). Whole-genome sequencing reveals recent and frequent genetic recombination between clonal lineages of *Cryphonectria parasitica* in western Europe. *Fungal Genetics and Biology* **130**, 122–133.

*Dennenmoser, S., Sedlazeck, F. J., Iwaszkiewicz, E., Li, X.-Y., Altmüller, J. & Nolte, A. W. (2017). Copy number increases of transposable elements and protein-coding genes in an invasive fish of hybrid origin. *Molecular Ecology* **26**(18), 4712–4724.

*Dennenmoser, S., Sedlazeck, F. J., Schatz, M. C., Altmüller, J., Zytnicki, M. & Nolte, A. W. (2019). Genome-wide patterns of transposon proliferation in an evolutionary young hybrid fish. *Molecular Ecology* **28**(6), 1491–1505.

*Di Prinzio, C. Y., Rossi, C. R., Ciancio, J., Garza, J. C. & Casaux, R. (2015). Disentangling the contributions of ocean ranching and net-pen aquaculture in the successful establishment of Chinook salmon in a Patagonian basin. *Environmental Biology of Fishes* **98**(9), 1987–1997.

Dlugosch, K. M., Anderson, S. R., Braasch, J., Cang, F. A. & Gillette, H. D. (2015). The devil is in the details: genetic variation in introduced populations and its contributions to invasion. *Molecular Ecology* **24**(9), 2095–2111.

Dlugosch, K. M. & Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology* **17**(1), 431–449.

Du, Z., Wu, Y., Chen, Z., Cao, L., Ishikawa, T., Kamitani, S., Sota, T., Song, F., Tian, L., Cai, W. & Li, H. (2021). Global phylogeography and invasion history of the spotted lanternfly revealed by mitochondrial phylogenomics. *Evolutionary Applications* **14**(4), 915–930.

Dueñas, M.-A., Hemming, D. J., Roberts, A. & Diaz-Soltero, H. (2021). The threat of invasive species to IUCN-listed critically endangered species: a systematic review. *Global Ecology and Conservation* **26**, e01476.

Dutech, C., Barrès, B., Bridier, J., Robin, C., Milgroom, M. G. & Ravigné, V. (2012). The chestnut blight fungus world tour: successive introduction events from diverse origins in an invasive plant fungal pathogen. *Molecular Ecology* **21**(16), 3931.

*Elfekih, S., Etter, P., Tay, W. T., Fumagalli, M., Gordon, K., Johnson, E. & Barro, P. D. (2018). Genome-wide analyses of the *Bemisia tabaci* species complex reveal contrasting patterns of admixture and complex demographic histories. *PLoS One* **13**(1), e0190555.

Errbii, M., Keilwagen, J., Hoff, K. J., Steffen, R., Altmüller, J., Oettler, J. & Schrader, L. (2021). Transposable elements and introgression introduce genetic variation in the invasive ant *Cardiocondyla obscurior*. *Molecular Ecology* **30**(23), 6211–6228.

Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M. & Facon, B. (2016). Is there a genetic paradox of biological invasion? *Annual Review of Ecology, Evolution, and Systematics* **47**(1), 51–72.

*Evans, J. D., McKenna, D., Scully, E., Cook, S. C., Dainat, B., Egekwu, N., Grubbs, N., Lopez, D., Lorenzen, M. D., Reyna, S. M., Rinkevich, F. D., Neumann, P. & Huang, Q. (2018). Genome of the small hive beetle (*Aethina tumida*, Coleoptera: Nitidulidae), a worldwide parasite of social bee colonies, provides insights into detoxification and herbivory. *GigaScience* **7**(12), giy138.

*Ewart, K. M., Griffin, A. S., Johnson, R. N., Kark, S., Magory Cohen, T., Lo, N. & Major, R. E. (2019). Two speed invasion: assisted and intrinsic dispersal of common mynas over 150 years of colonization. *Journal of Biogeography* **46**(1), 45–57.

*Eyer, P.-A., Blumenfeld, A. J. & Vargo, E. L. (2019). Sexually antagonistic selection promotes genetic divergence between males and females in an ant. *Proceedings of the National Academy of Sciences* **116**(48), 24157–24163.

Fauvergue, X., Vercken, E., Malausa, T. & Hufbauer, R. A. (2012). The biology of small, introduced populations, with special reference to biological control. *Evolutionary Applications* **5**(5), 5.

Fay, J. C. & Wu, C. I. (2000). Hitchhiking under positive Darwinian selection. *Genetics* **155**(3), 1405–1413.

Ferreira-Martins, D., Champer, J., McCauley, D. W., Zhang, Z. & Docker, M. F. (2021). Genetic control of invasive sea lamprey in the Great Lakes. *Journal of Great Lakes Research* **47**, S764–S775.

Fitzpatrick, M. C. & Keller, S. R. (2015). Ecological genomics meets community-level modelling of biodiversity: mapping the genomic landscape of current and future environmental adaptation. *Ecology Letters* **18**(1), 1.

Flanagan, B. A., Krueger-Hadfield, S. A., Murren, C. J., Nice, C. C., Strand, A. E. & Sotka, E. E. (2021). Founder effects shape linkage disequilibrium and genomic diversity of a partially clonal invader. *Molecular Ecology* **30**(9), 1962–1978.

Formenti, G., Theissinger, K., Fernandes, C., Bista, I., Bombarely, A., Bleidorn, C., Ciofi, C., Crottini, A., Godoy, J. A., Höglund, J., Malukiewicz, J., Mouton, A., Oomen, R. A., Paez, S., Palsbøll, P. J. & ET AL (2022). The era of reference genomes in conservation genomics. *Trends in Ecology & Evolution* **37**(3), 197–202.

Fortič, A., Rasheed, R. A.-S., Almajid, Z., Badreddine, A., Báez, J. C., Belmonte-Gallegos, Á., Bettoso, N., Borme, D., Camisa, F., Caracciolo, D., Çinar, M. E., Crocetta, F., Ćetković, I., Doğan, A., Galiya, M., ET AL. (2023). New records of introduced species in the Mediterranean Sea (April 2023).

Fournier, A., Penone, C., Pennino, M. G. & Courchamp, F. (2019). Predicting future invaders and future invasions. *Proceedings of the National Academy of Sciences* **116**(16), 7905–7910.

Garud, N. R. (2023). Understanding soft sweeps: a signature of rapid adaptation. *Nature Reviews Genetics* **24**(7), 420.

Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population-specific covariates. *Genetics* **201**(4), 1579.

*Gibson, M. J., Torres, M. d. L., Brandvain, Y. & Moyle, L. C. (2021). Introgression shapes fruit color convergence in invasive Galápagos tomato. *eLife* **10**, e64165.

Giraud, D., Lima, O., Rousseau-Gueutin, M., Salmon, A. & Aïnouche, M. (2021). Gene and transposable element expression evolution following recent and past polyploidy events in Spartina (Poaceae). *Frontiers in Genetics* **12**, 589160. https://doi.org/10.3389/fgene.2021.589160.

Goubert, C., Henri, H., Minard, G., Valiente Moro, C., Mavingui, P., Vieira, C. & Boulesteix, M. (2017). High-throughput sequencing of transposable element insertions suggests adaptive evolution of the invasive Asian tiger mosquito towards temperate environments. *Molecular Ecology* **26**(15), 3968–3981.

Gould, B. & Geber, M. (2016). Lack of adaptation from standing genetic variation despite the presence of putatively adaptive alleles in introduced sweet vernal grass (*Anthoxanthum odoratum*). *Journal of Evolutionary Biology* **29**(1), 178–187.

Gray, A. J. (1986). Do invading species have definable genetic characteristics? *Royal Society* **314**(1167), 655–674.

*Green, L., Apostolou, A., Faust, E., Palmqvist, K., Behrens, J. W., Havenhand, J. N., Leder, E. H. & Kvarnemo, C. (2021). Ancestral sperm ecotypes reveal multiple invasions of a non-native fish in northern Europe. *Cells* **10**(7), 1743.

Green, L., Faust, E., Hinchcliffe, J., Brijs, J., Holmes, A., Englund Örn, F., Svensson, O., Roques, J. A. C., Leder, E. H., Sandblom, E. & Kvarnemo, C. (2023). Invader at the edge—genomic origins and physiological differences of round gobies across a steep urban salinity gradient. *Evolutionary Applications* **16**(2), 321–337.

Grigoraki, L., Pipini, D., Labbé, P., Chaskopoulou, A., Weill, M. & Vontas, J. (2017). Carboxylesterase gene amplifications associated with insecticide resistance in *Aedes albopictus*: geographical distribution and evolutionary origin. *PLoS Neglected Tropical Diseases* **11**(4), e0005533.

Gutekunst, J., Andriantsoa, R., Falckenhayn, C., Hanna, K., Stein, W., Rasamy, J. & Lyko, F. (2018). Clonal genome evolution and rapid invasive spread of the marbled crayfish. *Nature Ecology & Evolution* **2**(3), 567–573.

Harvey-Samuel, T., Ant, T. & Alphey, L. (2017). Towards the genetic control of invasive species. *Biological Invasions* **19**(6), 6.

Helliwell, E. E., Faber-Hammond, J., Lopez, Z. C., Garoutte, A., von Wettberg, E., Friesen, M. L. & Porter, S. S. (2018). Rapid establishment of a flowering cline in *Medicago polymorpha* after invasion of North America. *Molecular Ecology* **27**(23), 4758–4774.

*Hernández, F., Brown, J. I., Kaminski, M., Harvey, M. G. & Lavretsky, P. (2021). Genomic evidence for rare hybridization and large demographic changes in the evolutionary histories of four North American dove species. *Animals* **11**(9), 2677.

Hernández, R. D., Kelley, J. L., Elyashiv, E., Melton, S. C., Auton, A., McVean, G., 1000 Genomes Project, Sella, G. & Przeworski, M. (2011). Classic selective sweeps were rare in recent human evolution. *Science (New York, N.Y.)* **331**(6019), 920–924. https://doi.org/10.1126/science.1198878.

Hessenauer, P., Fijarczyk, A., Martin, H., Prunier, J., Charron, G., Chapuis, J., Bernier, L., Tanguay, P., Hamelin, R. C. & Landry, C. R. (2020). Hybridization and introgression drive genome evolution of Dutch elm disease pathogens. *Nature Ecology & Evolution* **4**(4), 638.

Heuertz, M., Carvalho, S. B., Galindo, J., Rinkevich, B., Robakowski, P., Aavik, T., Altinok, I., Barth, J. M. I., Cotrim, H., Goessen, R., González-Martínez, S. C., Grebenc, T., Hoban, S., Kopatz, A., McMahon, B. J., et al. (2023). The application gap: genomics for biodiversity and ecosystem service management. *Biological Conservation* **278**, 109883.

Hiatt, D. & Flory, S. L. (2020). Populations of a widespread invader and co-occurring native species vary in phenotypic plasticity. *New Phytologist* **225**(1), 584–594.

Hoelzel, A. R. (2023). Where to now with the evolutionarily significant unit? *Trends in Ecology & Evolution* **38**(12), 1134.

Hodgins, K. A., Bock, D. G., Hahn, M. A., Heredia, S. M., Turner, K. G. & Rieseberg, L. H. (2015). Comparative genomics in the Asteraceae reveals little evidence for parallel evolutionary change in invasive taxa. *Molecular Ecology* **24**(9), 2226–2240.

*Hofmeister, N. R., Werner, S. J. & Lovette, I. J. (2021). Environmental correlates of genetic variation in the invasive European starling in North America. *Molecular Ecology* **30**(5), 1251–1263.

Hu, J., Chen, B. & Li, Z. (2014). Thermal plasticity is related to the hardening response of heat shock protein expression in two *Bactrocera* fruit flies. *Journal of Insect Physiology* **67**, 105–113.

Hübner, S., Sisou, D., Mandel, T., Todesco, M., Matzrafi, M. & Eizenberg, H. (2022). Wild sunflower goes viral: citizen science and comparative genomics allow tracking the origin and establishment of invasive sunflower in the Levant. *Molecular Ecology* **31**(7), 2061–2072.

*Hudson, J., Castilla, J. C., Teske, P. R., Beheregaray, L. B., Haigh, I. D., McQuaid, C. D. & Rius, M. (2021). Genomics-informed models reveal extensive stretches of coastline under threat by an ecologically dominant invasive species. *Proceedings of the National Academy of Sciences* **118**(23), e2022169118.

*Hudson, J., Johannesson, K., McQuaid, C. D. & Rius, M. (2020). Secondary contacts and genetic admixture shape colonization by an amphiatlantic epibenthic invertebrate. *Evolutionary Applications* **13**(3), 600–612.

Jaspers, C., Ehrlich, M., Pujolar, J. M., Künzel, S., Bayer, T., Limborg, M. T., Lombard, F., Browne, W. E., Stefanova, K. & Reusch, T. B. H. (2021). Invasion genomics uncover contrasting scenarios of genetic diversity in a widespread marine invader. *Proceedings of the National Academy of Sciences* **118**(51), e2116211118.

Jeffery, N. W., DiBacco, C., Van Wyngaarden, M., Hamilton, L. C., Stanley, R. R. E., Bernier, R., FitzGerald, J., Matheson, K., McKenzie, C. H., Nadukkalam Ravindran, P., Beiko, R. & Bradbury, I. R. (2017a). RAD sequencing reveals genomewide divergence between independent invasions of the European green crab (*Carcinus maenas*) in the Northwest Atlantic. *Ecology and Evolution* **7**(8), 2513–2524.

*Jeffery, N. W., DiBacco, C., Wringe, B. F., Stanley, R. R. E., Hamilton, L. C., Ravindran, P. N. & Bradbury, I. R. (2017b). Genomic evidence of hybridization between two independent invasions of European green crab (*Carcinus maenas*) in the Northwest Atlantic. *Heredity* **119**(3), 154.

Jeschke, J. M. (2014). General hypotheses in invasion ecology. *Diversity and Distributions* **20**(11), 1229–1234.

Kirkpatrick, M. & Barrett, B. (2015). Chromosome inversions, adaptive cassettes and the evolution of species' ranges. *Molecular Ecology* **24**(9), 2046.

Koch, J. B., Dupuis, J. R., Jardeleza, M.-K., Ouedraogo, N., Geib, S. M., Follett, P. A. & Price, D. K. (2020). Population genomic and phenotype diversity of invasive *Drosophila suzukii* in Hawai'i. *Biological Invasions* **22**(5), 1753–1770.

Konopiński, M. K., Fijarczyk, A. M. & Biedrzycka, A. (2023). Complex patterns shape immune genes diversity during invasion of common raccoon in Europe – selection in action despite genetic drift. *Evolutionary Applications* **16**(1), 134–151.

Konorov, E. A., Yurchenko, V., Patraman, I., Lukashev, A. & Oyun, N. (2021). The effects of genetic drift and genomic selection on differentiation and local adaptation of the introduced populations of *Aedes albopictus* in southern Russia. *PeerJ* **9**, e11776.

Kotsakiozi, P., Richardson, J. B., Pichler, V., Favia, G., Martins, A. J., Urbanelli, S., Armbruster, P. A. & Caccone, A. (2017). Population genomics of the Asian tiger mosquito, *Aedes albopictus*: insights into the recent worldwide invasion. *Ecology and Evolution* **7**(23), 10143–10157.

Kramer, I. M., Pfenninger, M., Feldmeyer, B., Dhimal, M., Gautam, I., Shrestha, P., Baral, S., Phuyal, P., Hartke, J., Magdeburg, A., Groneberg, D. A., Ahrens, B., Müller, R. & Waldvogel, A.-M. (2023). Genomic profiling of climate adaptation in *Aedes aegypti* along an altitudinal gradient in Nepal indicates nongradual expansion of the disease vector. *Molecular Ecology* **32**(2), 350–368.

Krehenwinkel, H., Rödder, D. & Tautz, D. (2015). Eco-genomic analysis of the poleward range expansion of the wasp spider *Argiope bruennichi* shows rapid adaptation and genomic admixture. *Global Change Biology* **21**(12), 4320–4332.

Krzemińska, U., Morales, H. E., Greening, C., Nyári, Á. S., Wilson, R., Song, B. K., Austin, C. M., Sunnucks, P., Pavlova, A. & Rahman, S. (2018). Population mitogenomics provides insights into evolutionary history, source of invasions and diversifying selection in the House Crow (*Corvus splendens*). *Heredity* **120**(4), 296–309.

Lacy, R. C. & Ballou, J. D. (1998). Effectiveness of selection in reducing the genetic load in populations of *Peromyscus polionotus* during generations of inbreeding. *Evolution; International Journal of Organic Evolution* **52**(3), 900.

Lande, R. & Shannon, S. (1996). The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution* **50**(1), 434–437.

Láruson, Á. J., Yeaman, S. & Lotterhos, K. E. (2020). The importance of genetic redundancy in evolution. *Trends in Ecology & Evolution* **35**(9), 809.

Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends in Ecology & Evolution* **17**(8), 386–391.

Lee, C. E. (2016). Evolutionary mechanisms of habitat invasions, using the copepod *Eurytemora affinis* as a model system. *Evolutionary Applications* **9**(1), 248.

Lee, C. E. & Gelembiuk, G. W. (2008). Evolutionary origins of invasive populations. *Evolutionary Applications* **1**(3), 427–448.

Lee, C. C. & Wang, J. (2018). Rapid expansion of a highly germline-expressed mariner element acquired through horizontal transfer in the fire ant genome. *Genome Biology and Evolution* **10**(12), 3262–3278.

*Lee, S. R. & Son, D. C. (2022). Genetic diversity pattern reveals the primary determinant of burcucumber (*Sicyos angulatus* L.) invasion in Korea. *Frontiers in Plant Science* **13**, 997521. https://doi.org/10.3389/fpls.2022.997521.

*Lee, Y., Schmidt, H., Collier, T. C., Conner, W. R., Hanemaaijer, M. J., Slatkin, M., Marshall, J. M., Chiu, J. C., Smartt, C. T., Lanzaro, G. C., Mulligan, F. S. & Cornel, A. J. (2019). Genome-wide divergence among invasive populations of *Aedes aegypti* in California. *BMC Genomics* **20**(1), 204.

*Lehnert, S. J., DiBacco, C., Jeffery, N. W., Blakeslee, A. M. H., Isaksson, J., Roman, J., Wringe, B. F., Stanley, R. R. E., Matheson, K., McKenzie, C. H., Hamilton, L. C. & Bradbury, I. R. (2018). Temporal dynamics of genetic clines of invasive European green crab (*Carcinus maenas*) in eastern North America. *Evolutionary Applications* **11**(9), 1656–1670.

Leydet, K. P., Grupstra, C. G. B., Coma, R., Ribes, M. & Hellberg, M. E. (2018). Host-targeted RAD-Seq reveals genetic changes in the coral *Oculina patagonica* associated with range expansion along the Spanish Mediterranean coast. *Molecular Ecology* **27**(11), 2529–2543.

Lin, Y., Chen, Y., Yi, C., Fong, J. J., Kim, W., Rius, M. & Zhan, A. (2017). Genetic signatures of natural selection in a model invasive ascidian. *Scientific Reports* **7**(1), 44080.

Liu, B., Yan, J., Li, W., Yin, L., Li, P., Yu, H., Xing, L., Cai, M., Wang, H., Zhao, M., Zheng, J., Sun, F., Wang, Z., Jiang, Z., Ou, Q., ET AL. (2020a). *Mikania micrantha* genome provides insights into the molecular mechanism of rapid growth. *Nature Communications* **11**(1), 340.

Liu, C., Zhang, Y., Ren, Y., Wang, H., Li, S., Jiang, F., Yin, L., Qiao, X., Zhang, G., Qian, W., Liu, B. & Fan, W. (2018). The genome of the golden apple snail *Pomacea canaliculata* provides insight into stress tolerance and invasive adaptation. *GigaScience* **7**(9), giy101.

Liu, G., Zhang, H., Zhao, C. & Zhang, H. (2020b). Evolutionary history of the toll-like receptor gene family across Vertebrates. *Genome Biology and Evolution* **12**(1), 3615.

Liu, Y., Henkel, J., Beaurepaire, A., Evans, J. D., Neumann, P. & Huang, Q. (2021). Comparative genomics suggests local adaptations in the invasive small hive beetle. *Ecology and Evolution* **11**(22), 15780–15791.

Liu, Z., Xing, L., Huang, W., Liu, B., Wan, F., Raffa, K. F., Hofstetter, R. W., Qian, W. & Sun, J. (2022). Chromosome-level genome assembly and population genomic analyses provide insights into adaptive evolution of the red turpentine beetle, Dendroctonus valens. *BMC Biology* **20**(1), 190.

Liu, Z., Sun, C., Qu, L., Wang, K. & Yang, N. (2016). Genome-wide detection of selective signatures in chicken through high density SNPs. *PLoS One* **11**(11), e0166146.

Lotterhos, K. E. & Whitlock, M. C. (2014). Evaluation of demographic history and neutral parameterization on the performance of FST outlier tests. *Molecular Ecology* **23**(9), 9.

Ma, L., Cao, L.-J., Hoffmann, A. A., Gong, Y.-J., Chen, J.-C., Chen, H.-S., Wang, X.-B., Zeng, A.-P., Wei, S.-J. & Zhou, Z.-S. (2020). Rapid and strong population genetic differentiation and genomic signatures of climatic adaptation in an invasive mealybug. *Diversity and Distributions* **26**(5), 610–622.

Mack, R. N., Simberloff, D., Mark Lonsdale, W., Evans, H., Clout, M. & Bazzaz, F. A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* **10**(3), 689–710.

Marsico, T. D., Burt, J. W., Espeland, E. K., Gilchrist, G. W., Jamieson, M. A., Lindström, L., Roderick, G. K., Swope, S., Szűcs, M. & Tsutsui, N. D. (2010). PERSPECTIVE: underutilized resources for studying the evolution of invasive species during their introduction, establishment, and lag phases. *Evolutionary Applications* **3**(2), 203–219.

*Marin, P., Jaquet, A., Picarle, J., Fablet, M., Merel, V., Delignette-Muller, M.-L., Ferrarini, M. G., Gibert, P. & Vieira, C. (2021). Phenotypic and transcriptomic responses to stress differ according to population geography in an invasive species. *Genome Biology and Evolution* **13**(9), evab208.

Martin, M. D., Olsen, M. T., Samaniego, J. A., Zimmer, E. A. & Gilbert, M. T. P. (2016). The population genomic basis of geographic differentiation in North American common ragweed (*Ambrosia artemisiifolia* L.). *Ecology and Evolution* **6**(11), 3760–3771.

Matheson, P. & McGaughran, A. (2022). Genomic data is missing for many highly invasive species, restricting our preparedness for escalating incursion rates. *Scientific Reports* **12**(1), 18937.

McGaughran, A., Dhami, M. K., Parvizi, E., Vaughan, A. L., Gleeson, D. M., Hodgins, K. A., Rollins, L. A., Tepolt, C. K., Turner, K. G., Atsawawaranunt, K., Battlay, P., Congrains, C., Crottini, A., Dennis, T. P. W., Lange, C., ET AL. (2024). Genomic tools in biological invasions: current state and future frontiers. *Genome Biology and Evolution* **16**(1), evad230.

Merilä, J. & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications* **7**(1), 1–14.

Mérel, V., Gibert, P., Buch, I., Rodriguez Rada, V., Estoup, A., Gautier, M., Fablet, M., Boulesteix, M. & Vieira, C. (2021). The worldwide invasion of *Drosophila suzukii* is accompanied by a large increase of transposable element load and a small number of putatively adaptive insertions. *Molecular Biology and Evolution* **38**(10), 4252–4267.

Mittan-Moreau, C. S., Kelehear, C., Toledo, L. F., Bacon, J., Guayasamin, J. M., Snyder, A. & Zamudio, K. R. (2022). Cryptic lineages and standing genetic variation across independent cane toad introductions. *Molecular Ecology* **31**(24), 6440–6456.

Molnar, J. L., Gamboa, R. L., Revenga, C. & Spalding, M. D. (2008). Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment* **6**(9), 485–492.

Moran, E. V. & Alexander, J. M. (2014). Evolutionary responses to global change: lessons from invasive species. *Ecology Letters* **17**(5), 637–649.

Mularo, A. J., Bernal, X. E. & DeWoody, J. A. (2022). Dominance can increase genetic variance after a population bottleneck: a synthesis of the theoretical and empirical evidence. *Journal of Heredity* **113**(3), 257–271.

Narum, S. R., Gallardo, P., Correa, C., Matala, A., Hasselman, D., Sutherland, B. J. G. & Bernatchez, L. (2017). Genomic patterns of diversity and divergence of two introduced salmonid species in Patagonia, South America. *Evolutionary Applications* **10**(4), 402–416.

Nei, M., Maruyama, T. & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution; International Journal of Organic Evolution* **29**(1), 1.

Nguyen, D., Rieu, I., Mariani, C. & van Dam, N. M. (2016). How plants handle multiple stresses: hormonal interactions underlying responses to abiotic stress and insect herbivory. *Plant Molecular Biology* **91**(6), 727–740.

Ni, P., Li, S., Lin, Y., Xiong, W., Huang, X. & Zhan, A. (2018). Methylation divergence of invasive Ciona ascidians: significant population structure and local environmental influence. *Ecology and Evolution* **8**(20), 10272–10287.

North, H. L., McGaughran, A. & Jiggins, C. D. (2021). Insights into invasive species from whole-genome resequencing. *Molecular Ecology* **30**(23), 6289–6308.

*Nørgaard, L. S., Mikkelsen, D. M. G., Elmeros, M., Chriél, M., Madsen, A. B., Nielsen, J. L., Pertoldi, C., Randi, E., Fickel, J., Brygida, S. & Ruiz-González, A. (2017). Population genomics of the raccoon dog (*Nyctereutes procyonoides*) in Denmark: insights into invasion history and population development. *Biological Invasions* **19**(5), 1637–1652.

Oggenfuss, U., Badet, T., Wicker, T., Hartmann, F. E., Singh, N. K., Abraham, L., Karisto, P., Vonlanthen, T., Mundt, C., McDonald, B. A. & Croll, D. (2021). A population-level invasion by transposable elements triggers genome expansion in a fungal pathogen. *eLife* **10**, e69249.

Oh, D., Kowalski, K. P., Quach, Q. N., Wijesinghege, C., Tanford, P., Dassanayake, M. & Clay, K. (2022). Novel genome characteristics contribute to the invasiveness of *Phragmites australis* (common reed). *Molecular Ecology* **31**(4), 1142–1159.

*Olazcuaga, L., Loiseau, A., Parrinello, H., Paris, M., Fraimout, A., Guedot, C., Diepenbrock, L. M., Kenis, M., Zhang, J., Chen, X., Borowiec, N., Facon, B., Vogt, H., Price, D. K., Vogel, H., ET AL. (2020). A whole-genome scan for association with invasion success in the fruit fly *Drosophila suzukii* using contrasts of allele frequencies corrected for population structure. *Molecular Biology and Evolution* **37**(8), 2369–2385.

Orr, H. A. (2005). The genetic theory of adaptation: a brief history. *Nature Reviews Genetics* **6**(2), 127.

*Ortego, J., Céspedes, V., Millán, A. & Green, A. J. (2021). Genomic data support multiple introductions and explosive demographic expansions in a highly invasive aquatic insect. *Molecular Ecology* **30**(17), 4189–4203.

Papanicolaou, A., Schetelig, M. F., Arensburger, P., Atkinson, P. W., Benoit, J. B., Bourtzis, K., Castañera, P., Cavanaugh, J. P., Chao, H., Childers, C., Curril, I., Dinh, H., Doddapaneni, H., Dolan, A., Dugan, S., ET AL. (2016). The whole genome sequence of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), reveals insights into the biology and adaptive evolution of a highly invasive pest species. *Genome Biology* **17**(1), 192.

Parker, J. D., Torchin, M. E., Hufbauer, R. A., Lemoine, N. P., Alba, C., Blumenthal, D. M., Bossdorf, O., Byers, J. E., Dunn, A. M., Heckman, R. W., Hejda, M., Jarošík, V., Kanarek, A. R., Martin, L. B., Perkins, S. E., ET AL. (2013). Do invasive species perform better in their new ranges? *Ecology* **94**(5), 985–994.

Parvizi, E., Dhami, M. K., Yan, J. & McGaughran, A. (2023). Population genomic insights into invasion success in a polyphagous agricultural pest, Halyomorpha halys. *Molecular Ecology* **32**(1), 138–151.

*Pearce, S. L., Clarke, D. F., East, P. D., Elfekih, S., Gordon, K. H. J., Jermiin, L. S., McGaughran, A., Oakeshott, J. G., Papanikolaou, A., Perera, O. P., Rane, R. V., Richards, S., Tay, W. T., Walsh, T. K., Anderson, A., ET AL. (2017). Genomic innovations, transcriptional plasticity and gene loss underlying the evolution and divergence of two highly polyphagous and invasive *Helicoverpa* pest species. *BMC Biology* **15**(1), 63.

Pélissié, B., Crossley, M. S., Cohen, Z. P. & Schoville, S. D. (2018). Rapid evolution in insect pests: the importance of space and time in population genomics studies. *Current Opinion in Insect Science* **26**, 8–16.

Pérez-Portela, R., Bumford, A., Coffman, B., Wedelich, S., Davenport, M., Fogg, A., Swenarton, M. K., Coleman, F., Johnston, M. A., Crawford, D. L. & Oleksiak, M. F. (2018). Genetic homogeneity of the invasive lionfish across the Northwest Atlantic and the Gulf of Mexico based on single nucleotide polymorphisms. *Scientific Reports* **8**(1) 1, 5062. https://doi.org/10.1038/s41598-018-23339-w.

Popovic, I., Bierne, N., Gaiti, F., Tanurdžić, M. & Riginos, C. (2021). Pre-introduction introgression contributes to parallel differentiation and contrasting

hybridization outcomes between invasive and native marine mussels. *Journal of Evolutionary Biology* **34**(1), 175–192.

Popovic, I. & Riginos, C. (2020). Comparative genomics reveals divergent thermal selection in warm- and cold-tolerant marine mussels. *Molecular Ecology* **29**(3), 519–535.

Promy, N. T., Newberry, M. & Gulisija, D. (2023). Rapid evolution of phenotypic plasticity in patchy habitats. *Scientific Reports* **13**(1), 1.

*Puckett, E. E., Park, J., Combs, M., Blum, M. J., Bryant, J. E., Caccone, A., Costa, F., Deinum, E. E., Esther, A., Himsworth, C. G., Keightley, P. D., Ko, A., Lundkvist, Å., McElhinney, L. M., Morand, S., et al. (2016). Global population divergence and admixture of the brown rat (*Rattus norvegicus*). *Proceedings of the Royal Society B: Biological Sciences* **283**(1841), 20161762.

Pyšek, P., Skálová, H., Cuda, J., Guo, W.-Y., Suda, J., Dolezal, J., Kauzál, O., Lambertini, C., Lučanová, M., Mandáková, T., Moravcová, L., Pyšková, K., Brix, H. & Meyerson, L. A. (2018). Small genome separates native and invasive populations in an ecologically important cosmopolitan grass. *Ecology* **99**(1), 79–90.

Qian, Z., Li, Y., Yang, J., Shi, T., Li, Z. & Chen, J. (2022). The chromosome-level genome of a free-floating aquatic weed *Pistia stratiotes* provides insights into its rapid invasion. *Molecular Ecology Resources* **22**(7), 2732–2743.

Rispe, C., Legeai, F., Nabity, P. D., Fernández, R., Arora, A. K., Baa-Puyoulet, P., Banfill, C. R., Bao, L., Barberà, M., Bouallègue, M., Bretaudeau, A., Brisson, J. A., Calevro, F., Capy, P., Catrice, O., et al. (2020). The genome sequence of the grape *Phylloxera* provides insights into the evolution, adaptation and invasion routes of an iconic pest. *BMC Biology* **18**(1), 90.

Rius, M., Bourne, S., Hornsby, H. G. & Chapman, M. A. (2015). Applications of next-generation sequencing to the study of biological invasions. *Current Zoology* **61**(3), 3.

Roe, A. D., Torson, A. S., Bilodeau, G., Bilodeau, P., Blackburn, G. S., Cui, M., Cusson, M., Doucet, D., Griess, V. C., Lafond, V., Paradis, G., Porth, I., Prunier, J., Srivastava, V., Tremblay, E., et al. (2019). Biosurveillance of forest insects: part I—integration and application of genomic tools to the surveillance of non-native forest insects. *Journal of Pest Science* **92**(1), 51–70.

*Rogério, F., Van Oosterhout, C., Ciampi-Guillardi, M., Correr, F. H., Hosaka, G. K., Cros-Arteil, S., Rodrigues Alves Margarido, G., Massola Júnior, N. S. & Gladieux, P. (2023). Means, motive and opportunity for biological invasions: genetic introgression in a fungal pathogen. *Molecular Ecology* **32**(10), 2428–2442.

Rollins, L. A., Moles, A. T., Lam, S., Buitenwerf, R., Buswell, J. M., Brandenburger, C. R., Flores-Moreno, H., Nielsen, K. B., Couchman, E., Brown, G. S., Thomson, F. J., Hemmings, F., Frankham, R. & Sherwin, W. B. (2013). High genetic diversity is not essential for successful introduction. *Ecology and Evolution* **3**(13), 4501–4517.

Roman, J. & Darling, J. A. (2007). Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology & Evolution* **22**(9), 454.

Rosenthal, W. C., McIntyre, P. B., Lisi, P. J., Prather, R. B. Jr., Moody, K. N., Blum, M. J., Hogan, J. D. & Schoville, S. D. (2021). Invasion and rapid adaptation of guppies (*Poecilia reticulata*) across the Hawaiian Archipelago. *Evolutionary Applications* **14**(7), 1747–1761.

Rosinger, H. S., Geraldes, A., Nurkowski, K. A., Battlay, P., Cousens, R. D., Rieseberg, L. H. & Hodgins, K. A. (2021). The tip of the iceberg: genome wide marker analysis reveals hidden hybridization during invasion. *Molecular Ecology* **30**(3), 810–825.

Salojärvi, J. (2019). Computational tools for population genomics. In *Population genomics: Concepts, Approaches and Applications* (ed. O. P. Rajora), pp. 127–160. Cham, Switzerland: Springer International Publishing.

*Schmidt, T. L., Chung, J., Honnen, A.-C., Weeks, A. R. & Hoffmann, A. A. (2020). Population genomics of two invasive mosquitoes (*Aedes aegypti* and *Aedes albopictus*) from the Indo-Pacific. *PLoS Neglected Tropical Diseases* **14**(7), e0008463.

Schwensow, N. I., Detering, H., Pederson, S., Mazzoni, C., Sinclair, R., Peacock, D., Kovaliski, J., Cooke, B., Fickel, J. & Sommer, S. (2017). Resistance to RHD virus in wild Australian rabbits: comparison of susceptible and resistant individuals using a genomewide approach. *Molecular Ecology* **26**(17), 4551–4561.

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pyšek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., et al. (2017). No saturation in the accumulation of alien species worldwide. *Nature Communications* **8**(1), 14435.

Selechnik, D., Richardson, M. F., Shine, R., DeVore, J. L., Ducatez, S. & Rollins, L. A. (2019). Increased adaptive variation despite reduced overall genetic diversity in a rapidly adapting invader. *Frontiers in Genetics* **10**, 1221.

*Shaw, E. C., Fowler, R., Ohadi, S., Bayly, M. J., Barrett, R. A., Tibbits, J., Strand, A., Willis, C. G., Donohue, K., Robeck, P. & Cousens, R. D. (2021). Explaining the worldwide distributions of two highly mobile species: *Cakile edentula* and *Cakile maritima*. *Journal of Biogeography* **48**(3), 603–615.

*Shen, J., Wang, Z., Su, Y. & Wang, T. (2021). Associations between population epigenetic differentiation and environmental factors in the exotic weed mile-a-minute (*Mikania micrantha*). *Weed Science* **69**(3), 307–332.

*Sherpa, S., Blum, M. G. B., Capblancq, T., Cumer, T., Rioux, D. & Després, L. (2019a). Unravelling the invasion history of the Asian tiger mosquito in Europe. *Molecular Ecology* **28**(9), 2360–2377.

Sherpa, S., Blum, M. G. B. & Després, L. (2019b). Cold adaptation in the Asian tiger mosquito's native range precedes its invasion success in temperate regions. *Evolution* **73**(9), 1793–1808.

Sherpa, S., Guéguen, M., Renaud, J., Blum, M. G. B., Gaude, T., Laporte, F., Akiner, M., Alten, B., Aranda, C., Barre-Cardi, H., Bellini, R., Bengoa Paulis, M., Chen, X.-G., Eritja, R., Flacio, E., et al. (2019c). Predicting the success of an invader: niche shift versus niche conservatism. *Ecology and Evolution* **9**(22), 12658–12675.

Sherpa, S., Rioux, D., Goindin, D., Fouque, F., François, O. & Després, L. (2018). At the origin of a worldwide invasion: unravelling the genetic makeup of the Caribbean bridgehead populations of the dengue vector *Aedes aegypti*. *Genome Biology and Evolution* **10**(1), 56–71.

Skrede, I., Murat, C., Hess, J., Maurice, S., Sønstebø, J. H., Kohler, A., Barry-Etienne, D., Eastwood, D., Högberg, N., Martin, F. & Kauserud, H. (2021). Contrasting demographic histories revealed in two invasive populations of the dry rot fungus *Serpula lacrymans*. *Molecular Ecology* **30**(12), 2772–2789.

Sotka, E. E., Baumgardner, A. W., Bippus, P. M., Destombe, C., Duermit, E. A., Endo, H., Flanagan, B. A., Kamiya, M., Lees, L. E., Murren, C. J., Nakaoka, M., Shainker, S. J., Strand, A. E., Terada, R., Valero, M., et al. (2018). Combining niche shift and population genetic analyses predicts rapid phenotypic evolution during invasion. *Evolutionary Applications* **11**(5), 781.

Sparks, J. T. & Dickens, J. C. (2017). Mini review: gustatory reception of chemicals affecting host feeding in aedine mosquitoes. *Pesticide Biochemistry and Physiology* **142**, 15–20.

Stern, D. B. & Lee, C. E. (2020). Evolutionary origins of genomic adaptations in an invasive copepod. *Nature Ecology & Evolution* **4**(8), 1084–1094.

Sultan, S. E., Horgan-Kobelski, T., Nichols, L. M., Riggs, C. E. & Waples, R. K. (2013). A resurrection study reveals rapid adaptive evolution within populations of an invasive plant. *Evolutionary Applications* **6**(2), 266.

*Tay, W. T., Rane, R. V., Padovan, A., Walsh, T. K., Elfekih, S., Downes, S., Nam, K., d'Alençon, E., Zhang, J., Wu, Y., Nègre, N., Kunz, D., Kriticos, D. J., Czepak, C., Otim, M. H. & Gordon, K. H. J. (2022). Global population genomic signature of *Spodoptera frugiperda* (fall armyworm) supports complex introduction events across the Old World. *Communications Biology* **5**(1), 297.

Taylor, P. J., Downs, S., Monadjem, A., Eiseb, S. J., Mulungu, L. S., Massawe, A. W., Mahlaba, T. A., Kirsten, F., Maltitz, E. V., Malebane, P., Makundi, R. H., Lamb, J. & Belmain, S. R. (2012). Experimental treatment-control studies of ecologically based rodent management in Africa: balancing conservation and pest management. *Wildlife Research* **39**(1), 51–61.

Tepolt, C. K. (2015). Adaptation in marine invasion: a genetic perspective. *Biological Invasions* **17**(3), 3.

*Tepolt, C. K. & Palumbi, S. R. (2015). Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader. *Molecular Ecology* **24**(16), 4145–4158.

Tepolt, C. K. & Palumbi, S. R. (2020). Rapid adaptation to temperature via a potential genomic Island of divergence in the invasive green crab, *Carcinus maenas*. *Frontiers in Ecology and Evolution* **8**, 580701. https://doi.org/10.3389/fevo.2020.580701.

Tepolt, C. K., Grosholz, E. D., de Rivera, C. E. & Ruiz, G. M. (2022). Balanced polymorphism fuels rapid selection in an invasive crab despite high gene flow and low genetic diversity. *Molecular Ecology* **31**(1), 55–69.

Teshima, K. M., Coop, G. & Przeworski, M. (2006). How reliable are empirical genomic scans for selective sweeps? *Genome Research* **16**(6), 702–712.

Theissinger, K., Fernandes, C., Formenti, G., Bista, I., Berg, P. R., Bleidorn, C., Bombarely, A., Crottini, A., Gallo, G. R., Godoy, J. A., Jentoft, S., Malukiewicz, J., Mouton, A., Oomen, R. A., Paez, S. & et al (2023). How genomics can help biodiversity conservation. *Trends in Genetics* **39**(7), 545–559.

*Thrimawithana, A. H., Wu, C., Christeller, J. T., Simpson, R. M., Hilario, E., Tooman, L. K., Begum, D., Jordan, M. D., Crowhurst, R., Newcomb, R. D. & Grapputo, A. (2022). The genomics and population genomics of the light brown apple moth, *Epiphyas postvittana*, an invasive tortricid pest of horticulture. *Insects* **13**(3), 264. https://doi.org/10.3390/insects13030264.

Tian, L., Song, T., He, R., Zeng, Y., Xie, W., Wu, Q., Wang, S., Zhou, X. & Zhang, Y. (2017). Genome-wide analysis of ATP-binding cassette (ABC) transporters in the sweetpotato whitefly, Bemisia tabaci. *BMC Genomics* **18**(1), 330.

*Tobias, P. A., Schwessinger, B., Deng, C. H., Wu, C., Dong, C., Sperschneider, J., Jones, A., Luo, Z., Zhang, P., Sandhu, K., Smith, G. R., Tibbits, J., Chagné, D. & Park, R. F. (2021). *Austropuccinia psidii*, causing myrtle rust, has a gigabase-sized genome shaped by transposable elements. *G3 Genes|Genomes|Genetics* **11**(3), jkaa015.

Travis, J. M. J. & Travis, E. R. (2002). Mutator dynamics in fluctuating environments. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **269**(1491), 591–597.

*Trucchi, E., Facon, B., Gratton, P., Mori, E., Stenseth, N. C. & Jentoft, S. (2016). Long live the alien: is high genetic diversity a pivotal aspect of crested porcupine (*Hystrix cristata*) long-lasting and successful invasion? *Molecular Ecology* **25**(15), 3527–3539.

Turelli, M. & Barton, N. H. (2006). Will population bottlenecks and multilocus epistasis increase additive genetic variance? *Evolution; International Journal of Organic Evolution* **60**(9), 1763–1776.

Uller, T. & Leimu, R. (2011). Founder events predict changes in genetic diversity during human-mediated range expansions. *Global Change Biology* **17**(11), 11.

Valencia-Montoya, W. A., Elfekih, S., North, H. L., Meier, J. I., Warren, I. A., Tay, W. T., Gordon, K. H. J., Specht, A., Paula-Moraes, S. V., Rane, R., Walsh, T. K. & Jiggins, C. D. (2020). Adaptive introgression across semipermeable species boundaries between local *Helicoverpa zea* and invasive *Helicoverpa armigera* moths. *Molecular Biology and Evolution* **37**(9), 2568–2583.

*Vallejo-Marín, M., Friedman, J., Twyford, A. D., Lepais, O., Ickert-Bond, S. M., Streisfeld, M. A., Yant, L., van Kleunen, M., Rotter, M. C. & Puzey, J. R. (2021). Population genomic and historical analysis suggests a global invasion by bridgehead processes in *Mimulus guttatus*. *Communications Biology* **4**(1), 327.

van Boheemen, L. A. & Hodgins, K. A. (2020). Rapid repeatable phenotypic and genomic adaptation following multiple introductions. *Molecular Ecology* **29**(21), 4102.

*van Boheemen, L. A., Lombaert, E., Nurkowski, K. A., Gauffre, B., Rieseberg, L. H. & Hodgins, K. A. (2017). Multiple introductions, admixture and bridgehead invasion characterize the introduction history of *Ambrosia artemisiifolia* in Europe and Australia. *Molecular Ecology* **26**(20), 5421–5434.

Vandepitte K. de Meyer T. Helsen K. van Acker K. Roldán-Ruiz I. Mergeay J. Honnay O. 2014 Rapid genetic adaptation precedes the spread of an exotic plant species *Molecular Ecology*, **23**(9), 2157

*Van Wallendael, A., Alvarez, M. & Franks, S. J. (2021). Patterns of population genomic diversity in the invasive Japanese knotweed species complex. *American Journal of Botany* **108**(5), 857–868.

*Vavassori, L., Honnen, A.-C., Saarman, N., Caccone, A. & Müller, P. (2022). Multiple introductions and overwintering shape the progressive invasion of *Aedes albopictus* beyond the Alps. *Ecology and Evolution* **12**(7), e9138.

Vera, M., Díez-del-Molino, D. & García-Marín, J.-L. (2016). Genomic survey provides insights into the evolutionary changes that occurred during European expansion of the invasive mosquitofish (*Gambusia holbrooki*). *Molecular Ecology* **25**(5), 1089–1105.

Vogel, H., Schmidtberg, H. & Vilcinskas, A. (2017). Comparative transcriptomics in three ladybird species supports a role for immunity in invasion biology. *Developmental & Comparative Immunology* **67**, 452–456.

Wagner, N. K., Ochocki, B. M., Crawford, K. M., Compagnoni, A. & Miller, T. E. X. (2017). Genetic mixture of multiple source populations accelerates invasive range expansion. *The Journal of Animal Ecology* **86**(1), 21.

*Wang, J., Gaughan, S., Lamer, J. T., Deng, C., Hu, W., Wachholtz, M., Qin, S., Nie, H., Liao, X., Ling, Q., Li, W., Zhu, L., Bernatchez, L., Wang, C. & Lu, G. (2020). Resolving the genetic paradox of invasions: preadapted genomes and postintroduction hybridization of bigheaded carps in the Mississippi River Basin. *Evolutionary Applications* **13**(2), 263–277.

Wang, P., Song, H., Li, C., Li, P., Li, A., Guan, H., Hou, L. & Wang, X. (2017). Genome-wide dissection of the heat shock transcription factor family genes in *Arachis*. *Frontiers in Plant Science* **8**, 106.

Wani, S. H., Choudhary, M., Barmukh, R., Bagaria, P. K., Samantara, K., Razzaq, A., Jaba, J., Ba, M. N. & Varshney, R. K. (2022). Molecular mechanisms, genetic mapping, and genome editing for insect pest resistance in field crops. *Theoretical and Applied Genetics* **135**(11), 3875–3895.

Wegner, K. M., Lokmer, A. & John, U. (2020). Genomic and transcriptomic differentiation of independent invasions of the pacific oyster *Crassostrea gigas*. *Frontiers in Ecology and Evolution* **8**, 567049. https://doi.org/10.3389/fevo.2020.567049.

Wellband, K. W., Pettitt-Wade, H., Fisk, A. T. & Heath, D. D. (2018). Standing genetic diversity and selection at functional gene loci are associated with differential invasion success in two non-native fish species. *Molecular Ecology* **27**(7), 1572–1585.

Willi, Y., Griffin, P. & Van Buskirk, J. (2013). Drift load in populations of small size and low density. *Heredity* **110**(3), 296–302.

Williamson, M. & Fitter, A. (1996). The varying success of invaders. *Ecology* **77**(6), 6.

Wolfe, L. M., Blair, A. C. & Penna, B. M. (2007). Does intraspecific hybridization contribute to the evolution of invasiveness?: an experimental test. *Biological Invasions* **9**(5), 5.

Wu, Z., Zhang, H., Bin, S., Chen, L., Han, Q. & Lin, J. (2016). Antennal and abdominal transcriptomes reveal chemosensory genes in the asian citrus psyllid, Diaphorina citri. *PLoS One* **11**(7), e0159372.

Wu, N., Zhang, S., Li, X., Cao, Y., Liu, X., Wang, Q., Liu, Q., Liu, H., Hu, X., Zhou, X. J., James, A. A., Zhang, Z., Huang, Y. & Zhan, S. (2019). Fall webworm genomes yield insights into rapid adaptation of invasive species. *Nature Ecology & Evolution* **3**(1), 1.

Yang, F., Crossley, M. S., Schrader, L., Dubovskiy, I. M., Wei, S.-J. & Zhang, R. (2022). Polygenic adaptation contributes to the invasive success of the Colorado potato beetle. *Molecular Ecology* **31**(21), 5568–5580.

Yang, M., He, Z., Huang, Y., Lu, L., Yan, Y., Hong, L., Shen, H., Liu, Y., Guo, Q., Jiang, L., Zhang, Y., Greenberg, A. J., Zhou, R., Ge, X., Wu, C.-I. & Shi, S. (2017). The emergence of the hyperinvasive vine, *Mikania micrantha* (Asteraceae), via admixture and founder events inferred from population transcriptomics. *Molecular Ecology* **26**(13), 3405–3423.

*Ye, Z., Williams, E., Zhao, C., Burns, C. W. & Lynch, M. (2021). The rapid, mass invasion of New Zealand by North American Daphnia "pulex". *Limnology and Oceanography* **66**(7), 2672–2683.

Yeaman, S. & Whitlock, M. C. (2011). The genetic architecture of adaptation under migration–selection balance. *Evolution* **65**(7), 1897.

Yin, X., Martinez, A. S., Sepúlveda, M. S. & Christie, M. R. (2021). Rapid genetic adaptation to recently colonized environments is driven by genes underlying life history traits. *BMC Genomics* **22**(1), 269.

Yoshida, K., Miyagi, R., Mori, S., Takahashi, A., Makino, T., Toyoda, A., Fujiyama, A. & Kitano, J. (2016). Whole-genome sequencing reveals small genomic regions of introgression in an introduced crater lake population of threespine stickleback. *Ecology and Evolution* **6**(7), 2190–2204.

*Yuan, X., Jiang, Y.-D., Wang, G.-Y., Yu, H., Zhou, W.-W., Liu, S., Yang, M.-F., Cheng, J., Gurr, G. M., Way, M. O. & Zhu, Z.-R. (2016). Odorant-binding proteins and chemosensory proteins from an invasive pest *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae). *Environmental Entomology* **45**(5), 1276–1286.

Zenetos, A. & Galanidi, M. (2020). Mediterranean non indigenous species at the start of the 2020s: recent changes. *Marine Biodiversity Records* **13**(1), 10.

Zhang, W., Yu, H., Lv, Y., Bushley, K. E., Wickham, J. D., Gao, S., Hu, S., Zhao, L. & Sun, J. (2020). Gene family expansion of pinewood nematode to detoxify its host defence chemicals. *Molecular Ecology* **29**(5), 940–955.

XI. SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Detailed methods.
Database S1. Searchable database containing query results.

Article 2 – Signatures of differential local adaptation indicate climate matching with specific native-range habitats in invasive raccoons (*Procyon lotor*)

1 Signatures of differential local adaptation indicate climate matching with specific native-

2 range habitats in invasive raccoons (*Procyon lotor*)

3 Running title: Raccoon local adaptations across ranges

4

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15

16   **Abstract**

17   Invasive species pose a major threat to ecosystems through habitat destruction,

18   disease transmission, and competition for resources. Invasion success depends on the

19   adaptive potential (i.e., the ability to respond to selection pressures that is dependent on the

20   levels of genetic variation) of the invading species. Here, we used reduced representation

21   sequencing of native and European invasive raccoon populations to identify putatively

22   adaptive changes across geographic regions. Our findings indicate that invasive raccoon

23   populations experienced a genome-wide reduction in genetic diversity; however, we

24   detected signatures of selection that were putatively related to local adaptation within the

25   invasive range. Analysis of loci under selection associated with either native or invasive

26   populations revealed parallel selection pressures between the invasive range and one of the

27   native populations, all inhabiting similar climatic niches. In contrast, the native population

28   from a different climatic region experienced distinct selective pressures. These results

29   highlight the importance of habitat similarity in invasion processes. They demonstrate that,

30   when predicting the potential range of an invasive species, it is not only the geographic

31   distribution of the native range that should be considered, but also the specific

32   characteristics of the habitat. Genomic regions under selection were linked to traits that

33   promote rapid expansion, including reproduction, cognitive function, stress response,

34   immunity, and energy production. Overall, this study illuminates how contemporary

35   selection in raccoons may accelerate the invasion process.

36

37   **Keywords:** biological invasions, adaptation, climate matching, native range, invasive range,

38   raccoon

39

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 among direct drivers of change in nature with the largest relative global impacts (Brondizio et al., 2019; Mack et al., 2000; Pyšek et al., 2020; Seebens et al., 2017), 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 genetic diversity after the invasion event (Dlugosch et al., 2015) or by bringing substantial levels of adaptive genetic diversity from their native range (Lee, 2016).

The detailed insight into invasion demography and its effect on genetic diversity enables the detection 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 characterized 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 populations to identify putatively adaptive changes among 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 Germany in the 1950s, where they spread into neighbouring countries and now inhabit a majority of Europe (Salgado, 2018). European populations were founded from at least four separate introductions, leaving a clear pattern in the mitochondrial markers (Fischer et al., 2015). Due to subsequent admixture of genetically divergent individuals, raccoons maintain relatively high overall genetic diversity (Biedrzycka et al., 2014). 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

88   found no decline in richness or stability of microbiome fauna among invasive populations

89   compared to what was found in native populations (Kołodziejczyk et al., 2025a), supporting

90   raccoon adaptability across different environments. Moreover, Konopiński et al. (2023)

91   revealed that the adaptive potential of invasive populations was retained in a set of

92   immune-related loci. The successful invasion of the raccoon throughout Europe is likely

93   aided by species-specific characteristics that may have a genetic basis, such as a high

94   reproductive rate (Ritke, 1990) and a diet based on currently available food resources

95   (Rulison et al., 2012). All these features could be potentially linked to functional genomic

96   diversity in regions related to reproduction, immunity or omnivory of both native and

97   invasive populations.

98   This study sought to analyze raccoon (*Procyon lotor*) populations from North America

99   (i.e., the native range) and Europe (i.e., the invasive range) to determine how these regions

100   have evolved independently over the past 75 years. The structure of invasive raccoon

101   populations has been well characterized, but the complex phylogeography and the vast

102   native range impair the identification of the exact source populations of the European

103   invasion (Cullingham et al., 2008). To mitigate this issue and to allow comparison of invasive

104   populations' genetic diversity with native populations from similar and different climatic

105   conditions, we analyzed two geographically and genetically distant native populations.

106   We aimed to identify specific local adaptations and relate them to those in the invasive

107   range. We expected to find signals of selection in specific SNPs by comparing overall

108   genomic diversity patterns with those found only at loci identified as outliers across all

109   populations studied. Additionally, we predicted that we would detect outlier loci different

110   from those that had previously been detected and were associated with immune–related

111   functions. Identification of such loci would serve as evidence for specific local adaptations.

112   Second, we aimed to identify SNP loci that could be directly associated with the raccoon

113   invasion in Europe. The presence of such outlier loci would indicate adaptive transitions

114   linked to the range shift. Furthermore, we predicted that comparing diversity patterns

115   among native populations inhabiting different environments and invasive populations would

116   reveal specific, habitat-related local adaptations. Genetic evidences supporting the role of

117   habitat matching between native and invasive ranges were discussed in relation to invasive

118   species management and the evolutionary process that leads to genomic differentiation

119   among geographic regions of invasive species.

120

121   **MATERIALS AND METHODS**

122   **Sample collection**

123   Between 2021 and 2023 we collected a total of 162 tissue samples from raccoons from their

124   native (near Melbourne, Florida (USA_F; n=26) and between Lincoln and Syracuse, Nebraska

125   (USA_N; n= 16)) and invasive range (Poland: The Warta Mouth National Park (PL_WM;

126   n= 30) and Kampinos National Park (PL_K; n= 9), Germany: Brandenburg (GE_B; n= 13),

127   Hessen (GE_H; n= 28) and Saxony (GE_S; n= 25) and Czech Republic (CZ; n= 15; Fig.1).

128   Samples from Florida were collected from adults transported to the Florida Wildlife Hospital,

129   Inc. during euthanasia or surgical anaesthesia directly after a vehicle collision, while samples

130   from Nebraska were collected as a result of local pest control programs. Tissue samples from

131   the invasive range were obtained during game activities in Poland, Germany and the Czech

132   Republic. All the samples were collected with sterile surgical blades and immediately stored

133   in 96% ethanol. Upon arrival at the laboratory, samples were stored at -20°C until DNA

134   extraction.

135

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 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).

Bioinformatic processing

Raw fastq files were demultiplexed with `process_radtags´ from the Stacks v2 software package (Rochette et al., 2019). Individual reads were trimmed using Trimmomatic v0.39 (Bolger et al., 2014) with the following parameters: pair-end mode with a maximum of two mismatches, 5 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,

43

159   GCA_015708975.1; (Tsuchiya et al., 2021) using Burrows-Wheeler Aligner (BWA) and `MEM

160   algorithm´ (Li, 2013) with default parameters.

161   SNP calling and PCR duplicate removal were performed using `gstacks´, from the

162   Stacks v2 software package. The `populations´ program was used for initial SNP filtering and

163   for generating input files for subsequent analyses. Parameters were set to 0.5 for maximum

164   observed heterozygosity and 4 for minimum minor allele count required to process a SNP, to

165   minimize false-positive SNPs. Further filtering was conducted in VCFtools v0.1.16 (Danecek

166   et al., 2011) with the following parameters: -minGQ 30, - minDP 10, -mac 3, -min-alleles 2, -

167   max-alleles 2 and -max-missing 0.95.

168

169   **Population structure analysis**

170   To generate baseline characteristics of genetic differentiation, we used two different

171   methods to resolve patterns of population structure. First, we used ADMIXTURE (Alexander

172   et al., 2009), a model-based clustering method. The optimal number of clusters was

173   determined using cross-validation (CV) error. Results were visualized using `ggplot2´

174   in R v4.4.2 (R Core Team, 2024). Then, we applied Principal Components Analysis (PCA)

175   as implemented in the function *glPCA* in the `adegenet´ package in R (Jombart, 2008).

176

177   **Genetic diversity**

178   To assess the level of genetic diversity in each cluster, diversity statistics, i.e. observed (H_O)

179   and expected heterozygosity (H_E), number of private alleles *(PA)*, percent of polymorphic loci

180   *(PPL)*, and heterozygosity deficit (F_{IS}) were calculated using the *populations* program in

181   Stacks v2 (Rochette et al., 2019). Nucleotide diversity (π) and Tajima's *D* were calculated

182   using a custom script in R available at https://github.com/konopinski/tajima/. To account for

183   differences in sample number between clusters, allelic richness (*AR*) was calculated using the

184   *allelic.richness* function in the `hierfstat´ R package (Goudet and Jombart, 2004)*.* To check

185   whether differences in diversity statistics between ranges were significant, a Wilcoxon rank-

186   sum test with continuity correction was performed using the *wilcox.test* function from `stats´

187   package in R.

188

189   **Patterns of selection**

190   We aimed to differentiate processes affecting genomic diversity that were primarily shaped

191   by demographic history from those influenced by local selection by identifying outlier SNPs

192   and comparing diversity between outlier and neutral loci. We identified global outliers, i.e.,

193   SNPs with frequencies deviating from neutrality, by comparing the genetic differentiation

194   among all studied populations. We used two complementary methods to minimize method-

195   specific bias and reduce false positives (Lotterhos et al., 2018). First, we used BayeScan (Foll,

196   2012) to identify differences in allele frequencies by comparing each population's allele

197   frequency to the common gene pool, using population-specific F_{ST} to highlight loci with

198   outlying signals. Posterior odds in BayeScan were set to 1 to reduce false positives. Second,

199   we used the `pcadapt´ R package to detect loci under selection by identifying outliers from

200   the pattern observed in principal component analysis (Luu et al., 2017). False positives were

201   filtered using Benjamini-Hochberg correction as implemented in the *qvalue* function from

202   the`qvalue´ R package (Storey et al., 2025). We then adopted a pooled approach, classifying

203   a SNP as an outlier for further analyses only if it was identified by both methods.

204   Since both BayeScan and PCAdapt detect global outliers, they do not identify specific

205   populations or population groups where selection occurs. Therefore, we calculated the C_2-

206   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, which are considered strong approximations of neutrality since all changes at the third position are synonymous (Künstner et al., 2011).

To get a more detailed insight into selection patterns present in both global and contrast outlier SNP sets, 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. The two diversity metrics were compared between the neutral SNP dataset and the global and contrast outliers for each population. We tested whether the means of the indices differ significantly between invasive and native populations using a Wilcoxon rank-sum test with a continuity correction as implemented in the *wilcox.test* R function from `stats´ package.

To address the uncertainty surrounding the identification of source populations of the invasive range, for the global outlier set, we traced the allele frequency pattern of loci that maintained non-zero frequencies in both native populations and at least two invasive ones. The presence of an allele in both the native and invasive ranges eliminates the possibility that it was removed by drift during invasion, thus the impact of demography, not selection, in shaping allele frequency. The similar allele frequencies in two genetically divergent native populations, coupled with a consistent pattern of allele frequencies across

231   invasive populations, potentially suggest specific selective pressures acting on these loci in

232   both ranges. To further untangle the selection pressure acting on outlier SNPs associated

233   with divergence between ranges, we searched for contrast outliers that exhibited common,

234   non-zero allele frequencies across ranges. Outlier SNPs with frequencies ranging from 0.2 to

235   0.8, further supported by Tajima's D values > 1, we treated as suggestive of balancing

236   selection.

237   Lastly, to predict the functions associated with putative outliers, we used SnpEff

238   (Cingolani et al., 2012). Genes associated with outlier SNPs were identified based on

239   scaffolds linked to specific effects derived from SnpEff annotations and grouped into three

240   main gene ontology (GO) domains: Biological Processes (BP), Molecular Functions (MF), and

241   Cellular Components (CC). Gene enrichment analysis was performed using the `topGO´

242   R package. Additionally, genes located on scaffolds containing outlier loci were identified

243   using InterProScan (Jones et al., 2014). Significant GO Terms (p < 0.05) were then filtered,

244   resulting in the database containing GO Terms annotated to at least 3 genes from the

245   background (all scaffolds) and at least 2 genes from the outliers set.

246

247   **RESULTS**

248   We initially obtained a dataset containing 5,200,166 SNPs. After filtering, the mean total

249   number of reads per individual was 5,112,216, ranging from 1,409,295 to 23,811,072.

250   The average depth of coverage was 92.5x, with a minimum of 25.4x and a maximum of

251   437.4x. Following the filtering pipeline, the final dataset used for all subsequent analysis

252   included 55,760 SNPs across 158 individuals (41 from the native and 117 from the invasive

253   range).

254

Population structure analysis

256 According to the analysis of cross-validation error in ADMIXTURE the optimal number of

257 clusters for the analysed dataset was K=4 (Fig. 2A). Individuals from the native populations in

258 the USA were either assigned to cluster 1 (Florida) or showed mixed assignment to clusters

259 1 and 2 (Nebraska). Cluster 2 was the primary assignment for all individuals from the Czech

260 Republic. Individuals from the other invasive populations were assigned to clusters 3 and 4,

261 with all individuals from Hessen (Germany) unequivocally assigned to cluster 3, Polish

262 populations (i.e. Warta Mouth National Park and Kampinos National Park) predominantly

263 assigned to cluster 4 and the individuals in the two remaining populations (i.e. Brandenburg

264 and Saxony, Germany) exhibiting mixed assignment to the two clades.

265   Principal Component Analysis (PCA) revealed a similar pattern (Fig. 2B). Populations

266 from the native range formed distinct clusters along PC1, with the Florida population

267 showing the greatest separation from the invasive populations. Individuals from the invasive

268 range were broadly distributed along the second principal component. When native

269 populations were excluded from the analysis, the Czech population appeared the most

270 differentiated among the invasive populations. The remaining individuals exhibited

271 a spatially structured distribution, with the Polish and Hessen populations forming the

272 outermost point clouds along the second principal component. The PCA results also

273 suggested gene flow or individuals exchange between the Brandenburg and Saxony

274 populations, consistent with their intermediate ancestry profiles in ADMIXTURE.

275 Consequently, these two populations were combined for downstream analyses (GE_B_S), as

276 were the two Polish populations (PL). Although PCA identified discrete clusters

277 corresponding to the Czech Republic, Hessen, and Polish populations, it also indicated

278   a gradient of genetic composition among invasive individuals, whereas more distinct

279   clustering was observed in the native range.

280

281   **Genetic diversity**

282   Native populations exhibited significantly greater (Wilcoxon rank-sum test - $p < 0.0001$)

283   estimates of nucleotide diversity (π), number of polymorphic loci (*PPL*), allelic richness (*AR*),

284   and observed (H_O) and expected heterozygosity (H_E) than those found in invasive

285   populations. The lowest values of diversity were observed for CZ population (Tab. 1). These

286   results indicated a general trend of higher genetic variation in native versus invasive

287   populations. Furthermore, a number of private alleles in the native populations was an order

288   of magnitude higher than in the invasive ones. All F_{IS} values were significantly different from

289   0 ($p < 0.001$). The highest F_{IS} estimated were observed in GE_B_S, PL and USA_F, indicating

290   slight heterozygote deficiency, which may be related to some level of population structure

291   within these genetic clusters, likely owing to a lack of closed populations and due to high

292   connectivity among raccoon populations (Tab. 1).

293

294   **Patterns of selection**

295   ***Global outliers***

296   Using the Bayescan and PCAdapt outlier detection methods, we identified over 3800 outlier

297   SNPs. However, only 92 of these SNPs were shared between both Bayescan and PCAdapt

298   and subsequently used in downstream analyses. Of these 92 SNPs, 16 were located in close

299   proximity to other outliers (ranging from 1 to 834 bp apart), suggesting potential linkage

300   among selected SNPs. This resulted in the identification of 76 regions potentially under

301   selection.

302   The values of Tajima's D and π in the neutral dataset (i.e. 4-fold SNPs) were primarily

303   centered around zero across all populations, providing a baseline reference for interpreting

304   the outlier dataset (Fig. 3A). In the set of global outliers, native populations (USA_F and

305   USA_N) exhibited higher Tajima's D estimates ($p < 0.0001$) and greater π ($p < 0.0001$)

306   in comparison with neutral dataset (Fig. 3B), with distributions shifted towards higher

307   values. In contrast, most outliers in the invasive range showed Tajima's D and π estimates

308   exhibited similar distribution as in case of 4-fold degenerate SNPs, nevertheless the

309   differences were significant in all, but CZ population (Fig. 3B, Tab. 2). Similarly, π values of

310   global outliers showed distribution resembling neutrality, but their means were significantly

311   divergent from those observed in neutral dataset (Tab. 2). Finally, among 24 loci that

312   maintained non-zero allele frequencies in both native populations and at least two invasive

313   populations, seven exhibited reduced frequencies in the populations from the continuous

314   part of the invasive range, while a balanced frequencies (ranging between 0.26 and 0.73) in

315   the native range.

316

317   ***C2-contrast statistics - between ranges outliers***

318   C2-contrast statistics were used to identify loci with outlying frequencies between ranges.

319   The 113 outliers identified using the C2-contrast did not overlap with global outliers and

320   exhibited a distinct pattern of Tajima's D and π estimates distribution (Fig. 3C). Contrast

321   outliers for USA_F native population and CZ invasive population exhibited a clear pattern of

322   neutral diversity in both Tajima's D and π estimates. In contrast, remaining invasive

323   populations and native USA_N exhibited similar distribution of Tajima's D and π. The

324   majority of outliers were characterized by high values of both estimators, typical for

325   balancing selection (Fig. 3C). The mean values of Tajima's D estimates in contrast outliers

326 were significantly higher in all but USA_F populations, in comparison with neutral dataset

327 (Tab. 3). Following the results of the similar distribution of Tajima's D and π estimates in the

328 native USA_N population and the invasive populations from the continuous invasion range

329 (GE_H, GE_B_S and PL), we examined allele frequencies and detected that the majority of

330 outlier SNPs (91) exhibited non-zero frequencies in these populations, and 46 out of them

331 exhibited balanced allele frequencies (0.2 – 0.8). Most of these loci exhibited zero (24 loci) or

332 low (20 loci with frequencies lower than 0.2) frequencies in the USA_F population.

333

334 ***Genes/regions under selection***

335 Out of the 92 global outlier SNPs, 34 were located in intergenic regions and were therefore

336 excluded from gene enrichment analysis. The remaining 58 outlier SNPs included 39 with

337 multiple predicted functional effects reflecting the presence of alternative gene models or

338 overlapping transcripts. Among the annotated SNPs, upstream, downstream and intronic

339 locations were predicted in 40, 23 and 46 SNPs, respectively. Additionally, two SNPs were

340 synonymous variants, one missense, and one was a stop-retained variant. The C2-contrast

341 statistic identified 113 outlier SNPs differentiating the native and invasive ranges, of which

342 96 were located in intergenic regions and therefore excluded from gene enrichment

343 analysis. Among the 113 remaining outlier SNPs, 67 were assigned to more than one

344 predicted functional category. Of the SNPs located within or proximal to genes, intronic,

345 downstream and upstream locations were predicted 105, 61, and 52 times, respectively;

346 14 synonymous, 7 missense, and 4 splice region variants were also predicted.

347 Among all global outlier SNPs, 44 were located in genes involved in immune

348 response, cognitive functions, tissue repair, and stress response processes potentially

349 contributing to local adaptation (Suppl. 2 Tab. 1). For the C2-contrast results, over one

350 hundred genes were identified, primarily associated with catalytic and enzymatic activity,

351 development, microtubules arrangement, inflammation process, nervous system, and

352 odorant detection (Suppl. 2 Tab. 2).

353   GO enrichment analysis of global outliers revealed 53 significantly enriched terms,

354 primarily associated with genes related to reproduction and hormonal pathways (e.g. female

355 gamete generation), intracellular metabolism (e.g. cytoplasm), protein and lipid binding

356 functions, immune functions (e.g. negative regulation of interferon-alpha production) and

357 neurological features (e.g. axon projection regeneration) (Fig. 5). For contrast outliers,

358 significantly enriched GO terms showed patterns similar to those found within the global

359 outlier dataset. Enriched terms were primarily related to the reproduction, development and

360 hormonal pathways (e.g. regulation of hormone levels, and embryonic limb morphogenesis),

361 muscle contraction and muscle tissue growth (e.g. microtubule-related), enzyme activity

362 (e.g. peroxidase), signalling pathways (e.g. transmembrane signalling receptor activity) and

363 neurological features (e.g. related to spinal cord) (Fig. 6).

364

365 **DISCUSSION**

366   In this study, we analysed raccoons from native and invasive ranges to investigate

367 genomic diversity, population structure and adaptation in a rapidly spreading invasive

368 mammal. Rapid adaptation is likely one of the mechanisms providing resilience to new

369 environments, enabling raccoons' expansion across Europe. Whether, and to what extent,

370 selection pressures are present in the invasive range depends on the extent of habitat

371 matching (Abellán et al., 2017; Redding et al., 2019) and is mediated by the amount of

372 standing genetic variation (Orr, 2005), existing phenotypic plasticity (Merilä and Hendry,

373 2014), and the demographic history of the founding individuals (Santos et al., 2012). Our

374   findings confirmed that invasive raccoon populations experienced a genome-wide reduction

375   in genetic diversity. Despite this loss, we detected signatures of local selection that are

376   putatively related to parallel climatic conditions within the invasive range and part of native

377   range. In contrast, the native population from a different climatic region experienced distinct

378   selective pressures. These results underscore the importance of habitat similarity in the

379   invasion process. Genomic regions under selection were linked to traits that promote rapid

380   expansion, including reproduction, cognitive function, stress response, immunity, and energy

381   production, showing that contemporary selection in raccoons may accelerate the invasion

382   process.

383

384   **Contrasting local patterns of selection**

385   Allele frequencies are influenced by both demographic process and local selection

386   (Willi et al., 2020). Comparing patterns across the genome in native and invasive ranges of

387   a species, and identifying loci that deviate from neutral expectations, can reveal adaptations

388   to the new environment (Hoban et al., 2016; Hodgins et al., 2025; North et al., 2021). In

389   contrast, loci located outside selection targets may be useful for identifying demographically

390   driven population diversity and substructure. For example, genomic signatures of local

391   adaptation related to an environmental stress response were detected in the tropical fruit

392   fly, *Bactrocera tryoni*, a pest currently undergoing intensive expansion in Australia (Parvizi

393   et al., 2024). Moreover, in an invasive population of Brown rat (*Rattus norvegicus*), outlier

394   loci identified as under selection were linked to lipid metabolism and immune function,

395   suggesting that these adaptations may have facilitated successful colonization of new

396   environments (Chen et al., 2021).

397   In this study, we first identified a set of global outlier loci and then characterized their

398   patterns of diversity in comparison to loci evolving under neutral expectations. The

399   distributions of Tajima's D and nucleotide diversity between neutral and global outlier loci

400   for native populations exhibited a strong shift towards higher values, suggesting balancing

401   selection acting on a substantial set of loci (balanced allele frequencies, high values of

402   Tajima's D and nucleotide diversity; Fig. 3A and 3B). This trend was not observed for invasive

403   populations, where only single loci showed signals of selection. We identified a small subset

404   of outlier SNPs (Fig. 3, bolded in Supplement 1) that displayed diversity patterns typical of

405   balancing selection in native populations but reduced frequencies in most invasive

406   populations, particularly those from the continuous part of the invasive range (GE_H,

407   GE_B_S, PL). Although the number of such loci is limited, their role may be

408   disproportionately important in maintaining multiple variants at a local scale and promoting

409   local adaptation. These findings may support the hypothesis that intermediate or variable

410   environments in the native range promote balancing selection, preserving high genetic

411   variation that serves as a substrate for directional selection in the invasive range and

412   counteracts the loss of polymorphisms due to genetic drift (Siewert and Voight, 2017).

413   Similarly, Stern and Lee, (2020) found a strong association between balancing selection in

414   the native range and parallel local evolution in the invaded range of the copepod

415   *Eurytemora affinis* complex. The invasive populations of this species respond to selection

416   through the parallel use of the same SNPs, to a much greater degree than expected. Similar

417   mechanisms have been observed in other species such as *Drosophila melanogaster,* and

418   European starling (Huang et al., 2016; Stuart et al., 2023).

419   One of the aims of this study was to identify SNPs located in genomic regions

420   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 analyzing

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* (Battlay, 2025). Our study

identified a large set of outlier SNPs potentially associated with divergence between ranges.

However, detailed inspection of allele frequency distributions revealed that, for most SNPs,

the contrast was caused by low allele frequencies in the native Florida population, while

balanced allele frequencies in native Nebraska and invasive populations from the continuous

part of the invasion range (GE_H, GE_B_S, PL). (Fig. 4, bolded in Supplement 1). This pattern

is consistent with that expected under 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). The distribution of Tajima D and π

values in outliers in CZ invasive population that resemble rather neutral pattern of diversity

may probably result from its generally lower genetic diversity that weaken the strength of

selection (Allendorf et al., 2013).

Although identification of source populations and exhaustive sampling in both ranges

is not necessary to detect allele frequency contrasts (Olazcuaga et al., 2020), such data

would improve the precision of assessing allele frequency changes in relation to invasion. 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

445 phylogeographic or population genetic study, and extensive translocations of individuals

446 across the native range (Cullingham et al., 2008). Additionally, in the European region, the

447 raccoon population is formed from multiple independent introductions followed by

448 subsequent admixture, further obscuring the geographic origins of the individuals that

449 contributed to the European invasion and reinforcing the need for cautious interpretation of

450 our findings.

451   To some extent, potential source populations can be inferred through ecological

452 niche similarity analyses (Sherpa and Després, 2021). Matching climate conditions between

453 native and invaded ranges may facilitate rapid parallel adaptation, enabling invasive

454 populations to re-establish adaptations to environmental characteristics present in their

455 native habitats. In our study, the two native populations differ substantially in their habitat

456 characteristics: Florida's subtropical and tropical climate is characterized by hot, humid

457 summers and mild winters, whereas Nebraska's climate is characterized by mean monthly

458 temperatures and rainfalls similar to those in central Europe. Therefore, the Nebraska

459 population is not just a closer genetic match to the European invasive populations (this

460 study) but is also a more appropriate proxy of the invasion source with regard to its habitat

461 characteristics. The identification of loci under parallel balancing selection between

462 Nebraska and the invasive populations underscores the importance of habitat matching in

463 shaping adaptive responses. These loci likely reflect adaptations to specific environmental

464 conditions shared between Nebraska and the European invasive range. Future studies

465 incorporating broader sampling across the native range and additional sampling from the

466 invasive range will be essential to further elucidate mechanisms underlying raccoon

467 adaptation during invasion.

468

469 **Enriched gene groups related to the raccoon's invasive potential**

470 The GO terms enrichment analysis of the genes associated with global and contrast outlier

471 SNPs revealed that the enriched gene groups are primarily related to reproduction,

472 hormonal pathways, neurological processes, cognitive functions, the locomotor system, and

473 environmental-related responses. These enriched functions point to environmental drivers

474 of divergence, but also may play a key role in facilitating raccoon invasion. Similar results

475 have been recently obtained in studies of other mammalian invaders. For example, in

476 invasive raccoon dogs (*Nyctereutes procyonoides*), enrichment was observed in genes

477 related to energy metabolism, locomotor system, detoxification and lipid metabolism (Lan et

478 al., 2022). Moreover, a study of the invasive brown rat (*Rattus norvegicus*) also identified the

479 enriched gene groups involved in fatty acid and lipid transport, as well as carbohydrates

480 binding, linking these findings to the success of species invasion (Chen et al., 2021). In both

481 sets of outliers we detected enrichment of GO terms related to reproduction, i.e. oogenesis

482 and female gamete generation, hormone regulation, embryonic development, and stress

483 response (Jessop et al., 2013). Globally, mammalian invasions are more likely in species with

484 larger and more frequent litters, and longer reproductive lifespans (Capellini et al., 2015).

485 Indeed, high reproductive rates were shown in Japanese raccoon populations (Ikeda et al.

486 2004). The difference in selection pressures, revealed by contrast analysis, between native

487 and invasive populations may reflect shifts in reproductive performance observed between

488 the Florida population occupying sub-tropical habitat and remaining populations. We also

489 observed enrichment in genes associated with neurological processes and various cognitive

490 functions which can support neuroplasticity and, consequently, facilitate adaptation to

491 a different environment (Axelrod et al., 2023). Cognitive capabilities, directly linked to

492 nervous system function (Frasnelli, 2021), have been shown to aid invasive species in

locating and adopting new food sources, and utilizing 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 among 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 enrichment of GO terms related to environmental response was found in both outlier sets. Most importantly, the functional enrichment in genes linked to mitochondrial cell respiration and oxidative stress response could suggest differential adaptation to cold and hot environments via influencing the metabolic rate (Sokolova, 2018; White et al., 2011; 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) suggests 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 suggests rapid adaptation (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).

517   Although we did not detect a direct overlap between SNPs identified as global and

518   contrast outliers, the results of GO enrichment analysis show substantial overlap in enriched

519   functions across both data sets (Supplement 3). While we cannot pinpoint the exact genes or

520   mechanisms involved in specific aspects of raccoon invasion, the outcomes of GO

521   enrichment analysis suggest that SNPs under selection may play a role in local adaptation to

522   the new range and in responses to novel environmental conditions. Interestingly, the results

523   here seem to suggest that selection is likely acting on functional outcomes more than

524   specific genetic alterations as the mechanism leading to local adaptive responses in the

525   invasive range. Similarly, the study on copy number variation in native and invasive

526   populations of the invasive weed *Ambrosia artemisiifolia* identified overrepresentation of

527   genes involved in environmental response and pathogen defence both in CNV windows and

528   SNP outliers (Wilson et al., 2025). Moreover, a recent study of the microbiome of invasive

529   raccoons revealed functional redundancy, where differing microbial communities

530   maintained similar functional roles (Kołodziejczyk et al., 2025b). Likewise, although specific

531   SNPs varied between global and contrast outlier methods, enriched functional categories

532   remained conserved, suggesting a parallel pattern of functional stability amid genetic

533   variation.

534

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

536   We detected a clear pattern of genetic differentiation between native and invasive raccoon

537   populations. Previous research has shown that European raccoon populations were

538   established through at least four separate introductions (Fischer et al., 2015), and the

539   presence of distinct genetic clusters remains evident in our results. However, the relatively

540   small genetic distances between invasive clusters suggest that, despite originating from

541 independent introductions, the founding individuals were genetically more similar to each

542 other than the two native populations analysed here. Most populations in the invasive range

543 belong to a continuum with an overlapping extent of the genetic clusters. The only isolated

544 cluster corresponds to the sampling site in the Czech Republic. As shown in previous studies

545 (Biedrzycka et al., 2019, 2014), this population is genetically isolated from other invasive

546 populations, exhibiting lower genetic diversity and no signs of expansion. The population

547 from Hessen in central Germany (GE_H), considered the primary introduction site in Europe,

548 also formed a homogenous cluster and exhibit reduced levels of diversity. According to the

549 central-marginal hypothesis of species expansion, asymmetric, core-to-edge gene flow

550 should be expected (Eckert et al., 2008). Here, this mechanism is supported by a high

551 proportion of assignment to the Hessian clade among genotypes in the population from

552 eastern Germany (GE_B_S). Further, due to lowered habitat quality and higher population

553 differentiation, lower genetic diversity should be visible across edge populations due to

554 reduced population sizes, greater isolation and drift. Such a pattern was detected in invasive

555 populations of invasive mealybug (Ma et al., 2020). In contrast to these expectations, we

556 detected higher levels of admixture and marginally higher genetic diversity in raccoons from

557 eastern Germany and Poland. In the case of raccoons, the decreased habitat quality does not

558 seem to be the case, as the species is known to thrive in a variety of habitats and use diverse

559 food sources (Rulison et al., 2012). As a result, raccoons spread eastwards in high numbers,

560 taking advantage of high habitat availability. The mixed support of the central-marginal

561 hypothesis was also detected in the cane toad invasion in Australia (Trumbo et al., 2016),

562 where the lack of a genetic diversity gradient was explained by habitat availability and

563 connectivity. Although it is predicted that asymmetrical gene flow from the centre of

564 a species' range may prevent adaptation of marginal populations and impact range

565   expansion (Bridle and Vines, 2007), the genetic diversity of raccoon marginal populations

566   suggests that local adaptation should not be dismissed.

567   The very consistent levels of genetic diversity detected in the two native populations,

568   despite large geographic distance and habitat dissimilarities between them, suggest that

569   they are a good representation of native range diversity. Raccoons from Nebraska were

570   more closely related to the European invasive populations, providing insight into the general

571   North American region that likely served as the source for the European invasion. The intra-

572   population diversity reflects patterns typically observed for invasive species. According to

573   a systematic review of genomic invasion studies, over a half of invasive populations

574   experienced a reduction of genetic diversity when compared to native populations, but in

575   most of all studied cases, the diversity was restored via admixture of divergent populations

576   in the invasive range (Kołodziejczyk et al., 2025a). Our results also add support to this rule;

577   although genetic variability within invasive populations was lower, they still maintained

578   a high number of polymorphic SNP loci.

579

580   **CONCLUSIONS AND IMPLICATIONS FOR INVASION MANAGEMENT**

581   In summary, our findings demonstrate that signatures of local selection differ

582   between native and invasive ranges and respond to different habitat characteristics.

583   Selection processes acting on genomic regions potentially linked to the raccoon's

584   exceptional performance appear more pronounced in native populations, as we detected

585   more loci showing selection signals in the native range. Nevertheless, the pervasive

586   influence of genetic drift has not entirely prevented local adaptation in invasive populations.

587   The contrasting allele frequencies of loci under selection between populations inhabiting

588   differentiated habitats, but not different ranges, underlines the role of habitat matching

589   during invasion. Although the hypothesis on the role of habitat similarity between native and

590   invasive ranges is central to explaining species invasions (Kolar and Lodge, 2001), it is rarely

591   confirmed by the analysis of genomic footprints of adaptation. Here, we showed that when

592   predicting the potential range of an invasive species, it is not only the geographic

593   distribution of the native range that should be considered, but also the specific

594   characteristics of the habitat. Our results demonstrate that conditions in the native range

595   may preadapt populations for similar habitat types in the invaded range. We highlight the

596   importance of habitat similarity in invasion processes, that is significant even for generalist

597   species such as the raccoon. Given that selection footprints were found in genomic regions

598   linked to traits such as reproduction, cognitive function, the stress response, immunity and

599   energy production, we demonstrated their role in facilitating rapid expansion. The raccoon is

600   currently invading almost the entire European continent (Salgado, 2018). It is predicted that

601   this invasion process will continue. Eradication of the species is unlikely, and management of

602   the invasion mainly relies on protecting native species and fragile habitats (Cunze et al.,

603   2025). While its broad climatic and habitat niche (Kochmann et al., 2021; Cunze et al., 2023)

604   suggests it can invade a wide variety of habitats, our findings can be used to prioritize

605   regions most vulnerable to invasion. The weakest selection signals detected in the least

606   diverse and isolated invasive population (CZ) prove that restricting gene flow between

607   invasive populations may limit their adaptive potential. Future research involving population

608   sampling along geographical gradients in both native and invasive ranges is needed to clarify

609   associations of outlier loci with environmental characteristics and to determine the

610   functional consequences of selection.

611

612

REFERENCES

Abellán, P., Carrete, M., Anadón, J. D., Cardador, L., Tella, J. L. (2017). Non-random patterns and temporal trends (1912-2012) in the transport, introduction and establishment of exotic birds in Spain and Portugal. *Diversity and Distributions* doi: 10.1111/ddi.12403

Alexander, D.H., Novembre, J., Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research* doi: 10.1101/gr.094052.109

Allendorf, F. W., Luikart, G. H., & Aitken, S. N. (2012). Conservation and the genetics of populations. *John Wiley & Sons*.

Axelrod, C.J., Gordon, S.P., Carlson, B.A. (2023). Integrating neuroplasticity and evolution. *Current Biology* doi: 10.1016/j.cub.2023.03.002

Battlay, P. (2025). Rapid parallel adaptation in distinct invasions of *Ambrosia artemisiifolia* is driven by large-effect structural variants. *Molecular Biology and Evolution* doi: 10.1093/molbev/msae270

Bayona-Vásquez, N.J., Glenn, T.C., Kieran, T.J., Pierson, T.W., Hoffberg, S.L., Scott, P.A., Bentley, K.E., Finger, J.W., Louha, S., Troendle, N., Diaz-Jaimes, P., Mauricio, R., Faircloth, B.C. (2019). Adapterama III: quadruple-indexed, double/triple-enzyme RADseq libraries (2RAD/3RAD). *PeerJ* doi: 10.7717/peerj.7724

Biedrzycka, A., Konopinski, M.K., Hoffman, E.A., Trujillo, A.L., Zalewski, A. (2019). Comparing raccoon MHC diversity in native and introduced ranges: evidence for the importance of functional immune diversity for adaptation and survival in novel environments. *Evolutionary Applications* doi: 10.1111/eva.12898

Biedrzycka, A., Zalewski, A., Bartoszewicz, M., Okarma, H., Jędrzejewska, E. (2014). The genetic structure of raccoon introduced in Central Europe reflects multiple invasion pathways. *Biological Invasions* doi: 10.1007/s10530-013-0595-8

Bolger, A.M., Lohse, M., Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* doi: 10.1093/bioinformatics/btu170

Bridle, J.R., Vines, T.H. (2007). Limits to evolution at range margins: when and why does adaptation fail? *Trends in Ecology & Evolution* doi: 10.1016/j.tree.2006.11.002

Brondizio, E.S., Settele, J., Díaz, S., Ngo, H.T. (2019). Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. *IPBES Secretariat*, Bonn.

Capellini, I., Baker, J., Allen, W.L., Street, S.E., Venditti, C. (2015). The role of life history traits in mammalian invasion success. *Ecology Letters* doi: 10.1111/ele.12493

Carneiro, V.C., Lyko, F. (2020). Rapid epigenetic adaptation in animals and its role in invasiveness. *Integrative and Comparative Biology* doi: 10.1093/icb/icaa023

Chen, Y., Zhao, L., Teng, H., Shi, C., Liu, Q., Zhang, J., Zhang, Y. (2021). Population genomics reveal rapid genetic differentiation in a recently invasive population of *Rattus norvegicus. Frontiers in Zoology* doi: 10.1186/s12983-021-00387-z

665 Chow, P.K.Y., Lurz, P.W.W., Lea, S.E.G. (2018). A battle of wits? Problem-solving abilities in
666   invasive eastern grey squirrels and native Eurasian red squirrels. *Animal Behaviour* doi:
667   10.1016/j.anbehav.2017.12.022

668 Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., Land, S.J., Lu, X., Ruden,
669   D.M. (2012). A program for annotating and predicting the effects of single nucleotide
670   polymorphisms, SnpEff. *Fly,* Austin doi: 10.4161/fly.19695

671 Cullingham, C.I., Kyle, C.J., Pond, B.A., White, B.N. (2008). Genetic structure of raccoons in
672   eastern North America based on mtDNA: implications for subspecies designation and
673   rabies disease dynamics. *Canadian Journal of Zoology* doi: 10.1139/Z08-072

674 Cunze, S., Klimpel, S., Kochmann, J. (2023). Land cover and climatic conditions as potential
675   drivers of the raccoon (*Procyon lotor*) distribution in North America and Europe.
676   *European journal of wildlife research* doi: 10.1007/s10344-023-01679-x

677 Cunze, S., Schneider, G., Peter, N. and Klimpel, S., (2025). Linking patterns to processes: Using
678   hunting bag data to classify raccoon (*Procyon lotor*) invasion stages in Germany since
679   the 2000s. *Ecological Indicators* doi: 10.1016/j.ecolind.2025.113568

680 Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E.,
681   Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 1000 Genomes Project
682   Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics* doi:
683   10.1093/bioinformatics/btr330

684 DeCoursey, T.E. (2013). Voltage-gated proton channels: molecular biology, physiology, and
685   pathophysiology of the HV family. *Physiological Reviews* doi:
686   10.1152/physrev.00011.2012

687 Dlugosch, K.M., Anderson, S.R., Braasch, J., Cang, F.A., Gillette, H.D. (2015). The devil is in the
688   details: genetic variation in introduced populations and its contributions to invasion.
689   *Molecular Ecology* doi: 10.1111/mec.13183

690 Dlugosch, K.M., Parker, I.M. (2008). Founding events in species invasions: genetic variation,
691   adaptive evolution, and the role of multiple introductions. *Molecular Ecology* doi:
692   10.1111/j.1365-294X.2007.03538.x

693 Early, R., Bradley, B.A., Dukes, J.S., Lawler, J.J., Olden, J.D., Blumenthal, D.M., Gonzalez, P.,
694   Grosholz, E.D., Ibañez, I., Miller, L.P., Sorte, C.J.B., Tatem, A.J. (2016). Global threats
695   from invasive alien species in the twenty-first century and national response capacities.
696   *Nature Communications* doi: 10.1038/ncomms12485

697 Eckert, C.G., Samis, K.E., Lougheed, S.C. (2008). Genetic variation across species' geographical
698   ranges: the central–marginal hypothesis and beyond. *Molecular Ecology* doi:
699   10.1111/j.1365-294X.2007.03659.x

700 Fischer, M.L., Hochkirch, A., Heddergott, M., Schulze, C., Anheyer-Behmenburg, H.E., Lang, J.,
701   Michler, F.-U., Hohmann, U., Ansorge, H., Hoffmann, L., Klein, R., Frantz, A.C. (2015).
702   Historical invasion records can be misleading: genetic evidence for multiple
703   introductions of invasive raccoons (*Procyon lotor*) in Germany. *PLOS ONE* doi:
704   10.1371/journal.pone.0125441

705 Foll, M. (2012). *BayeScan v2.0 User Manual.*

706 Frasnelli, E. (2021). Evolution and function of neurocognitive systems in non-human animals. *Scientific Reports* doi: 10.1038/s41598-021-02736-8

708 Gautier, M. (2015). Genome-wide scan for adaptive divergence and association with population-specific covariates. *Genetics* doi: 10.1534/genetics.115.181453

710 Giska, I., Pimenta, J., Farelo, L., Boursot, P., Hackländer, K., Jenny, H., Reid, N., Montgomery, W.I., Prodöhl, P.A., Alves, P.C., Melo-Ferreira, J. (2022). The evolutionary pathways for local adaptation in mountain hares. *Molecular Ecology* doi: 10.1111/mec.16338

713 Goudet, J., Jombart, T. (2004). *hierfstat: Estimation and Tests of Hierarchical F-Statistics.* doi: 10.32614/CRAN.package.hierfstat

715 Griffin, A.S., Peneaux, C., Machovsky-Capuska, G.E., Guez, D. (2022). How alien species use cognition to discover, handle, taste, and adopt novel foods. *Current Opinion in Behavioral Sciences* doi: 10.1016/j.cobeha.2022.101136

718 Hoban, S., Kelley, J.L., Lotterhos, K.E., Antolin, M.F., Bradburd, G., Lowry, D.B., Poss, M.L., Reed, L.K., Storfer, A., Whitlock, M.C. (2016). Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. *The American Naturalist* doi: 10.1086/688018

722 Hodgins, K.A., Battlay, P., Bock, D.G. (2025). The genomic secrets of invasive plants. *New Phytologist* doi: 10.1111/nph.20368

724 Huang, Y., Tran, I., Agrawal, A.F. (2016). Does genetic variation maintained by environmental heterogeneity facilitate adaptation to novel selection? *The American Naturalist* doi: 10.1086/686889

727 Ikeda, T., Asano, M., Matoba, Y., Abe, G. (2004). Present status of invasive alien raccoon and its impact in Japan. *Global Environmental Research*

729 Jasper, R.J., Yeaman, S. (2024). Local adaptation can cause both peaks and troughs in nucleotide diversity within populations. *G3: Genes|Genomes|Genetics* doi: 10.1093/g3journal/jkae225

732 Jernelöv, A. (2017). Raccoons in Europe (Germany). In: Jernelöv, A. (Ed.), The long-term fate of invasive species: aliens forever or integrated immigrants with time? Springer International Publishing, Cham, pp. 217–230. doi: 10.1007/978-3-319-55396-2_15

735 Jessop, T.S., Letnic, M., Webb, J.K., Dempster, T. (2013). Adrenocortical stress responses influence an invasive vertebrate's fitness in an extreme environment. *Proceedings of the Royal Society B: Biological Sciences* doi: 10.1098/rspb.2013.1444

738 Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* doi: 10.1093/bioinformatics/btn129

740 Jones, P., Binns, D., Chang, H.-Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H., Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A.F., Sangrador-Vegas, A., Scheremetjew, M., Yong, S.-Y., Lopez, R., Hunter, S. (2014). InterProScan 5: genome-scale protein function classification. *Bioinformatics* doi: 10.1093/bioinformatics/btu031

744 Kawecki, T.J., Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters* doi: 10.1111/j.1461-0248.2004.00684.x

746 Kochmann, J., Cunze, S., Klimpel, S. (2021). Climatic niche comparison of raccoons Procyon
747   lotor and raccoon dogs *Nyctereutes procyonoides* in their native and non-native ranges.
748   *Mammal review* doi: 10.1111/mam.12249

749 Kolar, C.S., Lodge, D.M. (2001). Progress in invasion biology: predicting invaders. *Trends in*
750   *Ecology & Evolution* doi: 10.1016/S0169-5347(01)02101-2

751 Kołodziejczyk, J., Fijarczyk, A., Porth, I., Robakowski, P., Vella, N., Vella, A., Kloch, A.,
752   Biedrzycka, A. (2025a). Genomic investigations of successful invasions: the picture
753   emerging from recent studies. *Biological Reviews* doi: 10.1111/brv.70005

754 Kołodziejczyk, J., Hoffman, E., Biedrzycka, A. (2025b). Native and invasive raccoon
755   populations host distinct microbiomes that may facilitate invasion. *NeoBiota* doi:
756   10.3897/neobiota.101.157515

757 Konopiński, M.K., Fijarczyk, A.M., Biedrzycka, A. (2023). Complex patterns shape immune
758   genes diversity during invasion of common raccoon in Europe - Selection in action
759   despite genetic drift. *Evolutionary Applications* doi: 10.1111/eva.13517

760 Künstner, A., Nabholz, B., Ellegren, H. (2011). Significant selective constraint at 4-fold
761   degenerate sites in the avian genome and its consequence for detection of positive
762   selection. *Genome Biology and Evolution* doi: 10.1093/gbe/evr112

763 Lan, T., Li, H., Yang, S., Shi, M., Han, L., Sahu, S.K., Lu, Y., Wang, J., Zhou, M., Liu, H., Huang, J.,
764   Wang, Q., Zhu, Y., Wang, L., Xu, Y., Lin, C., Liu, H., Hou, Z. (2022). The chromosome-scale
765   genome of the raccoon dog: insights into its evolutionary characteristics. *iScience* doi:
766   10.1016/j.isci.2022.105117

767 Lande, R., Shannon, S. (1996). The role of genetic variation in adaptation and population
768   persistence in a changing environment. *Evolution* doi: 10.1111/j.1558-
769   5646.1996.tb04504.x

770 Le Luyer, J., Laporte, M., Beacham, T.D., Kaukinen, K.H., Withler, R.E., Leong, J.S., Rondeau,
771   E.B., Koop, B.F., Bernatchez, L. (2017). Parallel epigenetic modifications induced by
772   hatchery rearing in a Pacific salmon. *Proceedings of the National Academy of Sciences*
773   doi: 10.1073/pnas.1711229114

774 Lee, C.E. (2016). Evolutionary mechanisms of habitat invasions, using the copepod
775   *Eurytemora affinis* as a model system. *Evolutionary Applications* doi:
776   10.1111/eva.12334

777 Leigh, D.M., Lischer, H.E.L., Guillaume, F., Grossen, C., Günther, T. (2021). Disentangling
778   adaptation from drift in bottlenecked and reintroduced populations of Alpine ibex.
779   *Molecular Ecology Resources* doi: 10.1111/1755-0998.13442

780 Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-
781   MEM. doi: 10.48550/arXiv.1303.3997

782 Liebl, A. (2013). *Physiological and behavioral mechanisms of range expansion in the house*
783   *sparrow (Passer domesticus).* University of South Florida, Tampa Graduate Theses and
784   Dissertations.

785 Lotterhos, K.E., Yeaman, S., Degner, J., Aitken, S., Hodgins, K.A. (2018). Modularity of genes
786   involved in local adaptation to climate despite physical linkage. *Genome Biology* doi:
787   10.1186/s13059-018-1545-7

788 Luu, K., Bazin, E., Blum, M.G.B. (2017). pcadapt: an R package to perform genome scans for
789   selection based on principal component analysis. *Molecular Ecology Resources* doi:
790   10.1111/1755-0998.12592

791 Ma, L., Cao, L.-J., Hoffmann, A.A., Gong, Y.-J., Chen, J.-C., Chen, H.-S., Wang, X.-B., Zeng, A.-P.,
792   Wei, S.-J., Zhou, Z.-S. (2020). Rapid and strong population genetic differentiation and
793   genomic signatures of climatic adaptation in an invasive mealybug. *Diversity and*
794   *Distributions* doi: 10.1111/ddi.13053

795 Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., Bazzaz, F.A. (2000). Biotic
796   invasions: causes, epidemiology, global consequences, and control. *Ecological*
797   *Applications* doi: 10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2

798 Malishev, M., Kramer-Schadt, S. (2021). Movement, models, and metabolism: individual-
799   based energy budget models as next-generation extensions for predicting animal
800   movement outcomes across scales. *Ecological Modelling* doi:
801   10.1016/j.ecolmodel.2020.109413

802 Merilä, J., Hendry, A.P. (2014). Climate change, adaptation, and phenotypic plasticity: the
803   problem and the evidence. *Evolutionary Applications* doi: 10.1111/eva.12137

804 Molnar, J.L., Gamboa, R.L., Revenga, C., Spalding, M.D. (2008). Assessing the global threat of
805   invasive species to marine biodiversity. *Frontiers in Ecology and the Environment* doi:
806   10.1890/070064

807 Mounger, J., Ainouche, M.L., Bossdorf, O., Cavé-Radet, A., Li, B., Parepa, M., Salmon, A., Yang,
808   J., Richards, C.L. (2021). Epigenetics and the success of invasive plants. *Philosophical*
809   *Transactions of the Royal Society B: Biological Sciences* doi: 10.1098/rstb.2020.0117

810 North, H.L., McGaughran, A., Jiggins, C.D. (2021). Insights into invasive species from whole-
811   genome resequencing. *Molecular Ecology* doi: 10.1111/mec.15999

812 Oh, D., Kowalski, K.P., Quach, Q.N., Wijesinghege, C., Tanford, P., Dassanayake, M., Clay, K.
813   (2022). Novel genome characteristics contribute to the invasiveness of *Phragmites*
814   *australis* (common reed). *Molecular Ecology* doi: 10.1111/mec.16293

815 Olazcuaga, L., Loiseau, A., Parrinello, H., Paris, M., Fraimout, A., Guedot, C., Diepenbrock,
816   L.M., Kenis, M., Zhang, J., Chen, X., Borowiec, N., Facon, B., Vogt, H., Price, D.K., Vogel,
817   H., Prud'homme, B., Estoup, A., Gautier, M. (2020). A whole-genome scan for
818   association with invasion success in the fruit fly *Drosophila suzukii* using contrasts of
819   allele frequencies corrected for population structure. *Molecular Biology and Evolution*
820   doi: 10.1093/molbev/msaa098

821 Orr, H.A. (2005). The genetic theory of adaptation: a brief history. *Nature Reviews Genetics*
822   doi: 10.1038/nrg1523

823 Parvizi, E., Vaughan, A.L., Dhami, M.K., McGaughran, A. (2024). Genomic signals of local
824   adaptation across climatically heterogeneous habitats in an invasive tropical fruit fly
825   (*Bactrocera tryoni*). *Heredity* doi: 10.1038/s41437-023-00657-y

826 Pritchard, J.K., Di Rienzo, A. (2010). Adaptation – not by sweeps alone. *Nature Reviews*
827   *Genetics* doi: 10.1038/nrg2880

828 Pyšek, P., Hulme, P.E., Simberloff, D., Bacher, S., Blackburn, T.M., Carlton, J.T., Dawson, W.,
829   Essl, F., Foxcroft, L.C., Genovesi, P., Jeschke, J.M., Kühn, I., Liebhold, A.M., Mandrak,
830   N.E., Meyerson, L.A., Pauchard, A., Pergl, J., Roy, H.E., Seebens, H., van Kleunen, M.,
831   Vilà, M., Wingfield, M.J., Richardson, D.M. (2020). Scientists' warning on invasive alien
832   species. *Biological Reviews* doi: 10.1111/brv.12627

833 Redding, D. W., Pigot, A. L., Dyer, E. E., Şekercioğlu, Ç. H., Kark, S., Blackburn, T. M. (2019).
834   Location-level processes drive the establishment of alien bird populations worldwide.
835   *Nature* doi: 10.1038/s41586-019-1292-2

836 Ritke, M.E. (1990). Quantitative assessment of variation in litter size of the raccoon *Procyon*
837   *lotor*. *American Midland Naturalist* doi: 10.2307/2426567

838 Rochette, N.C., Rivera-Colón, A.G., Catchen, J.M. (2019). Stacks 2: Analytical methods for
839   paired-end sequencing improve RADseq-based population genomics. *Molecular*
840   *Ecology* doi: 10.1111/mec.15253

841 Rollins, L.A., Moles, A.T., Lam, S., Buitenwerf, R., Buswell, J.M., Brandenburger, C.R., Flores-
842   Moreno, H., Nielsen, K.B., Couchman, E., Brown, G.S., Thomson, F.J., Hemmings, F.,
843   Frankham, R., Sherwin, W.B. (2013). High genetic diversity is not essential for successful
844   introduction. *Ecology and Evolution* doi: 10.1002/ece3.824

845 Rulison, E.L., Luiselli, L., Burke, R.L. (2012). Relative impacts of habitat and geography on
846   raccoon diets. *American Midland Naturalist* doi: 10.1674/0003-0031-168.2.231

847 Salgado, I. (2018). Is the raccoon (*Procyon lotor*) out of control in Europe? *Biodiversity and*
848   *Conservation* doi: 10.1007/s10531-018-1535-9

849 Santos, J., Pascual, M., Simões, P., Fragata, I., Lima, M., Kellen, B., Santos, M., Marques, A.,
850   Rose, M.R., Matos, M. (2012). From nature to the laboratory: the impact of founder
851   effects on adaptation. *Journal of Evolutionary Biology* doi: 10.1111/jeb.12008

852 Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S.,
853   Pyšek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C.,
854   Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jäger, H., Kartesz, J., Kenis,
855   M., Kreft, H., Kühn, I., Lenzner, B., Liebhold, A., Mosena, A., Moser, D., Nishino, M.,
856   Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli,
857   S., Roy, H.E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., van Kleunen, M.,
858   Walker, K., Weigelt, P., Yamanaka, T., Essl, F. (2017). No saturation in the accumulation
859   of alien species worldwide. *Nature Communications* doi: 10.1038/ncomms14435

860 Sheldon, E.L., Schrey, A., Andrew, S.C., Ragsdale, A., Griffith, S.C. (2018). Epigenetic and
861   genetic variation among three separate introductions of the house sparrow (*Passer*
862   *domesticus*) into Australia. *Royal Society Open Science* doi: 10.1098/rsos.172185

863 Sherpa, S., Després, L. (2021). The evolutionary dynamics of biological invasions: A multi-
864   approach perspective. *Evolutionary Applications* doi: 10.1111/eva.13215

865 Siewert, K.M., Voight, B.F. (2017). Detecting long-term balancing selection using allele
866   frequency correlation. *Molecular Biology and Evolution* doi: 10.1093/molbev/msx209

867 Sokolova, I. (2018). Mitochondrial adaptations to variable environments and their role in
868　　　animals' stress tolerance. *Integrative and Comparative Biology* doi: 10.1093/icb/icy017

869 Stange, M., Barrett, R.D.H., Hendry, A.P. (2021). The importance of genomic variation for
870　　　biodiversity, ecosystems and people. *Nature Reviews Genetics* doi: 10.1038/s41576-
871　　　020-00288-7

872 Stern, D.B., Lee, C.E. (2020). Evolutionary origins of genomic adaptations in an invasive
873　　　copepod. *Nature Ecology & Evolution* doi: 10.1038/s41559-020-1201-y

874 Storey, J.D., Bass, A.J., Dabney, A., Robinson, D. (2025). *qvalue: Q-value estimation for false*
875　　　*discovery rate control*. doi:10.18129/B9.bioc.qvalue, R package version
876　　　2.42.0, https://bioconductor.org/packages/qvalue.

877 Stuart, K.C., Hofmeister, N.R., Zichello, J.M., Rollins, L.A. (2023). Global invasion history and
878　　　native decline of the common starling: insights through genetics. *Biological Invasions*
879　　　doi: 10.1007/s10530-022-02982-5

880 Teixeira, J.C., Huber, C.D. (2021). The inflated significance of neutral genetic diversity in
881　　　conservation genetics. *Proceedings of the National Academy of Sciences* doi:
882　　　10.1073/pnas.2015096118

883 Trumbo, D.R., Epstein, B., Hohenlohe, P.A., Alford, R.A., Schwarzkopf, L., Storfer, A. (2016).
884　　　Mixed population genomics support for the central marginal hypothesis across the
885　　　invasive range of the cane toad (*Rhinella marina*) in Australia. *Molecular Ecology* doi:
886　　　10.1111/mec.13754

887 Tsuchiya, M.T.N., Dikow, R.B., Koepfli, K.-P., Frandsen, P.B., Rockwood, L.L., Maldonado, J.E.
888　　　(2021). Whole-genome sequencing of procyonids reveals distinct demographic
889　　　histories in kinkajou (*Potos flavus*) and northern raccoon (*Procyon lotor*). *Genome*
890　　　*Biology and Evolution* doi: 10.1093/gbe/evaa255

891 White, C.R., Alton, L.A., Frappell, P.B. (2011). Metabolic cold adaptation in fishes occurs at
892　　　the level of whole animal, mitochondria and enzyme. *Proceedings of the Royal Society*
893　　　*B: Biological Sciences* doi: 10.1098/rspb.2011.2060

894 Willi, Y., Fracassetti, M., Bachmann, O., Van Buskirk, J. (2020). Demographic processes linked
895　　　to genetic diversity and positive selection across a species' range. *Plant*
896　　　*Communications* doi: 10.1016/j.xplc.2020.100111

897 Wollenberg Valero, K.C., Garcia-Porta, J., Irisarri, I., Feugere, L., Bates, A., Kirchhof, S.,
898　　　Jovanović Glavaš, O., Pafilis, P., Samuel, S.F., Müller, J., Vences, M., Turner, A.P., Beltran-
899　　　Alvarez, P., Storey, K.B. (2022). Functional genomics of abiotic environmental
900　　　adaptation in lacertid lizards and other vertebrates. *Journal of Animal Ecology* doi:
901　　　10.1111/1365-2656.13617

902 **TABLES**

903 Tab. 1. Genetic diversity estimates in invasive (CZ, GE_B_S, GE_H and PL) and native
904 populations (USA_F and USA_N). Studied sites: 55760. *Pop* - population, *N* - number of
905 analysed samples, *PA* - number of private alleles, *PPL* – percent of polymorphic loci, *N gen* -
906 mean number of individuals genotyped per locus in population, H_O – observed
907 heterozygosity, H_E – expected heterozygosity, *AR* – allelic richness, F_{IS} - inbreeding
908 coefficient, *π*1000* – nucleotide diversity multiplied by a thousand. Values of F_{IS} significantly
909 different from 0 were marked with asterisks.

910

Pop	N	PA	PPL	N gen	H_O	H_E	AR	F_{IS}	π*1000
CZ	15	288	45.9	14.8	0.140	0.141	1.435	0.019*	1.382
GE_B_S	36	266	65.5	34.8	0.157	0.170	1.561	0.047*	1.973
GE_H	28	230	50.9	27.7	0.149	0.150	1.457	0.012*	1.534
PL	38	259	61.6	36.4	0.144	0.154	1.508	0.039*	1.856
USA_F	25	5992	70.3	24.6	0.180	0.185	1.629	0.029*	2.117
USA_N	16	2209	70.7	15.7	0.181	0.180	1.647	0.016*	2.129

911

912 Tab. 2. Results of Wilcoxon rank sum test with continuity correction of Tajima's *D* and
913 nucleotide diversity (π) values between the neutral and global outliers dataset for invasive
914 (CZ, GE_B_S, GE_H and PL) and native populations (USA_F and USA_N).
915

| Pop | Tajima's *D* | | π | |
	W	p-value	W	p-value
CZ	28172	0.369	32104	<0.001
GE_B_S	30629	0.022	37232	<0.001
GE_H	31512	0.003	37306	<0.001
PL	33092	<0.001	35180	<0.001
USA_F	13456	<0.001	15396	<0.001
USA_N	19065	<0.001	22078	0.0065

916

917

918 Tab. 3. Results of Wilcoxon rank sum test with continuity correction of Tajima's *D* and
919 nucleotide diversity (π) values between the neutral and contrast outliers dataset for invasive
920 (CZ, GE_B_S, GE_H and PL) and native populations (USA_F and USA_N).
921

	Tajima's *D*		π	
Pop	**W**	**p-value**	**W**	**p-value**
CZ	38731	0.002	26661	<0.001
GE_B_S	13763	<0.001	15221	<0.001
GE_H	17726	<0.001	18348	<0.001
PL	19522	<0.001	18805	<0.001
USA_F	33120	0.958	39414	<0.001
USA_N	21182	<0.001	19966	<0.001

922

923

FIGURES

926 Fig. 1. Geographic locations of studied populations: native (left side) and invasive (right

927 side).

928

929 **A**

930

931 **B**

932
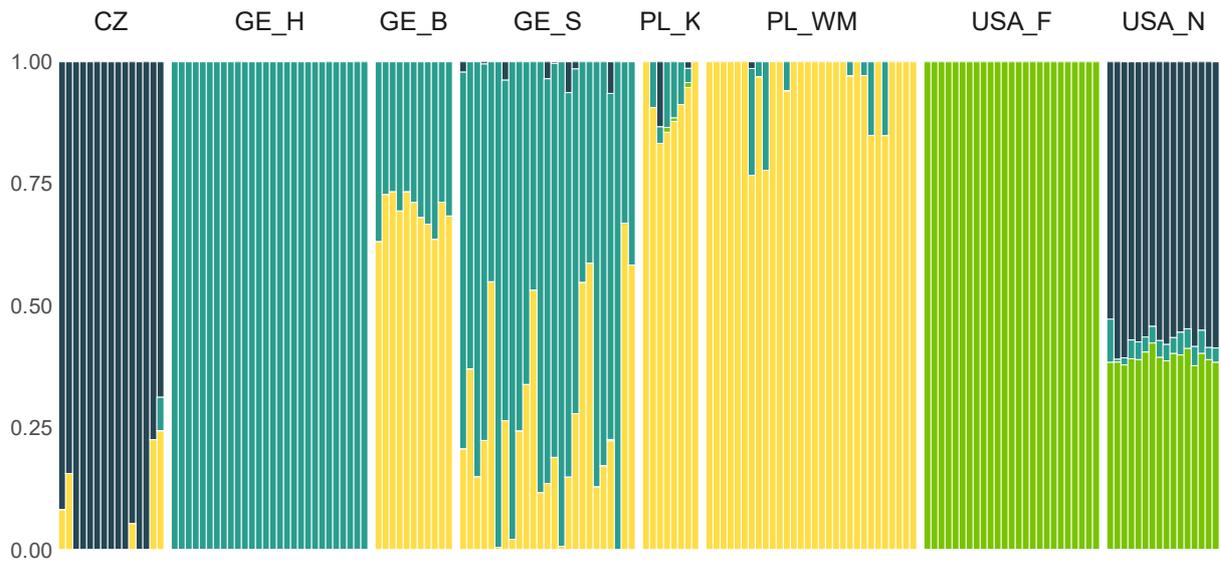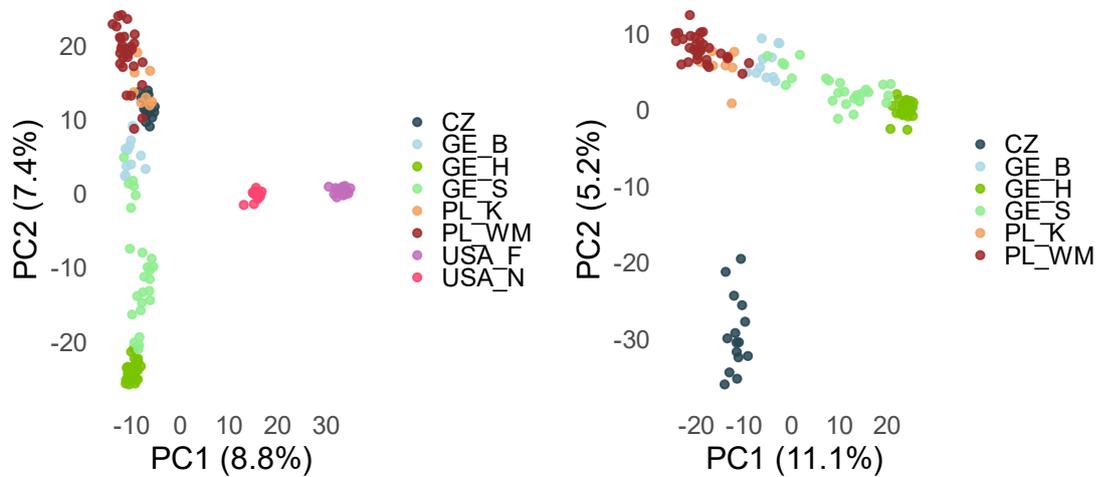
Fig. 2. Clustering of the analysed individuals based on A) ADMIXTURE results at K = 4, B) Principal component analysis (PCA). PCA plots are shown for all studied populations (left) and for invasive populations only (right).
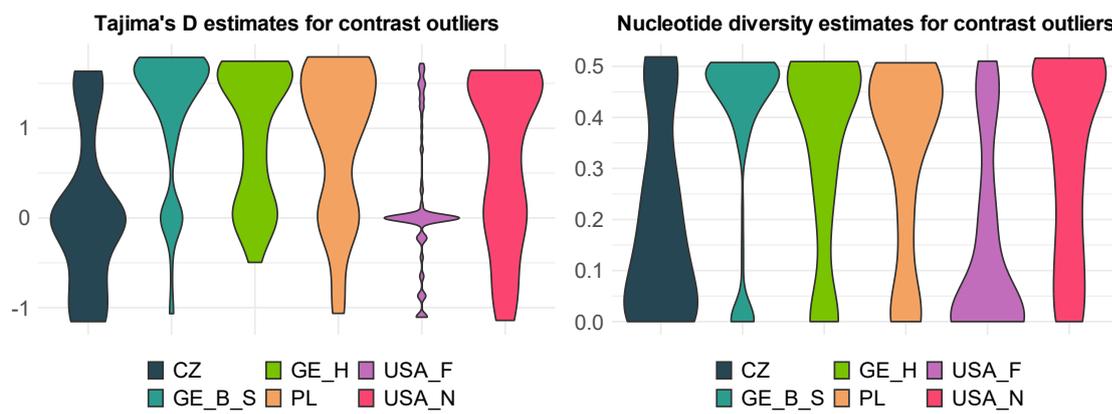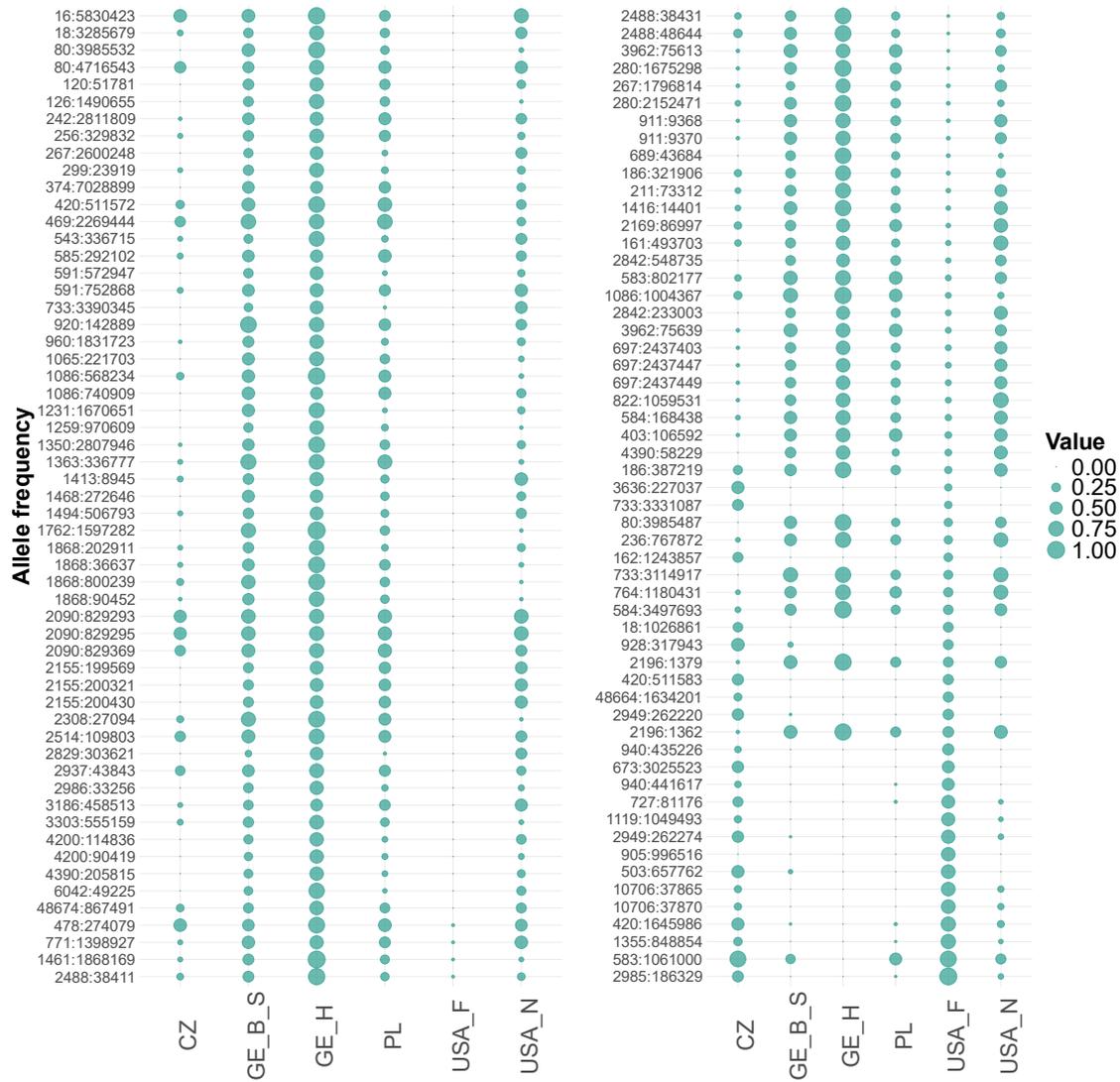
936

937   **A**
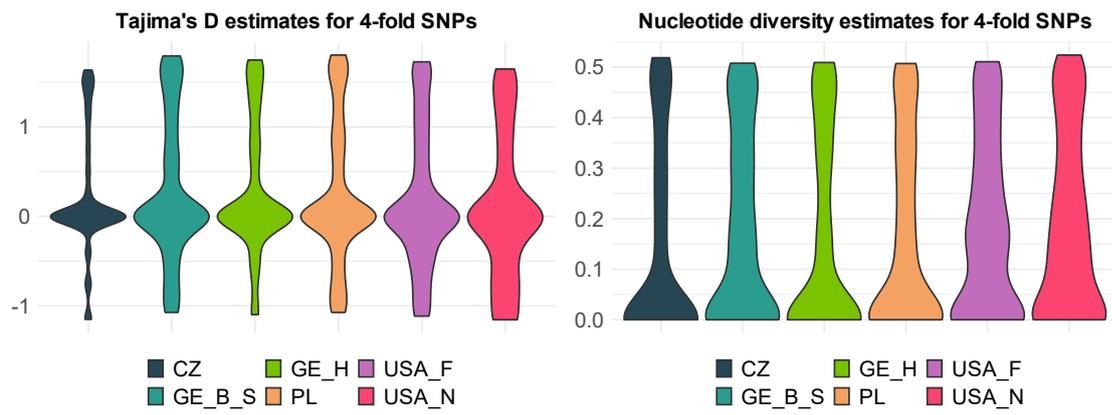
938

939   **B**
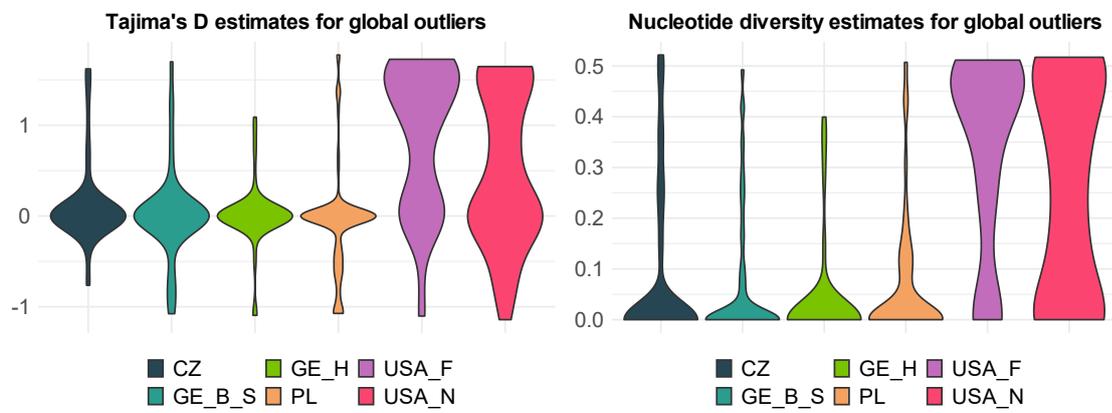
940

941   **C**

942

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

946 Fig. 4. Allele frequencies of contrast outliers across all studied populations. IDs represent the
947 abridged name of chromosome and position (CHR:POS) of SNPs according to the raccoon
948 genome assembly Plotor_platanus500 (Tsuchiya et al., 2021).

949
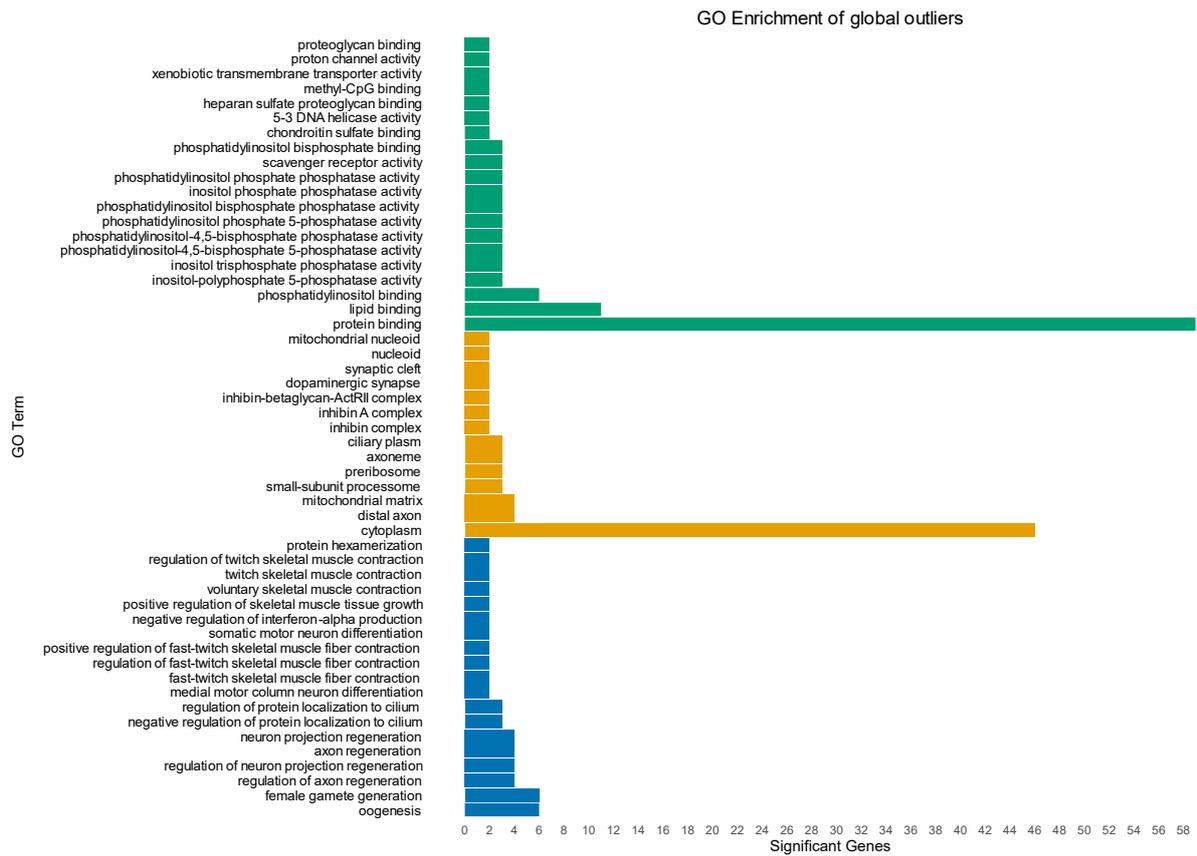
Fig. 5. Top 15 GO Enrichment groups (Green – molecular functions, orange – cellular components, blue – biological process) across global outliers. The detailed list of terms and definitions is in Supplement 3.

953

954
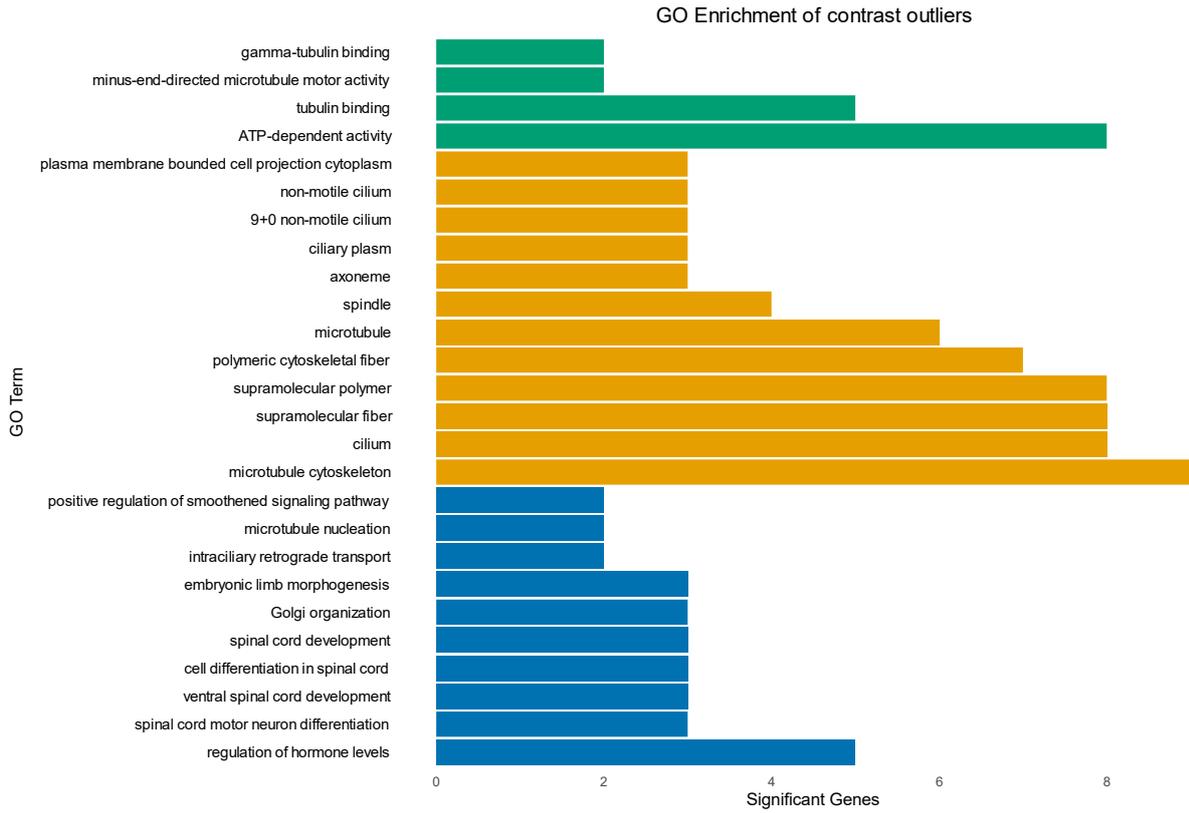
Fig. 6. Top 15 GO Enrichment groups (Green – molecular functions, orange – cellular components, blue – biological process) across outliers detected by C2-contrast statistics. The detailed list of terms and definitions is in Supplement 3.

955

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959   **SUPPLEMENT MATERIALS:**

960   1) Supplement_1 – Allele frequencies, Tajima's D and nucleotide diversity estimates

961   for global and contrast outliers

962   https://docs.google.com/spreadsheets/d/1hirz1cczCinrncyNsEdkrl8NED6IREDl/edit?usp=d

963   rive_link&ouid=10720391185317937758&rtpof=true&sd=true

964   2) Supplement_2 – Genes in which global and contrast outlier SNPs were identified,

965   and their functions

966   https://docs.google.com/document/d/1CmU-xKHC8jRWwhXfO0mf8PvUT-

967   iWRd3Q/edit?usp=drive_link&ouid=10720391185317937758&rtpof=true&sd=true

968   3) Supplement_3 – Gene Ontology Terms and definitions for genes in which global

969   and contrast outliers were identified

970   https://docs.google.com/spreadsheets/d/1g7I5AUOoAAq1am8YH4Y38OuaZs4csXqF/edit?

971   usp=drive_link&ouid=10720391185317937758&rtpof=true&sd=true

Article 3 – Native and invasive raccoon populations host distinct microbiomes that may facilitate invasion

Kołodziejczyk, J., Hoffman, E., Biedrzycka, A. (2025). Native and invasive raccoon populations host distinct microbiomes that may facilitate invasion. NeoBiota 101: 45-71. https://doi.org/10.3897/neobiota.101.157515

IF 2024 – 3.0

Points assigned to the journal by the Ministry of Education and Science – 140

Research Article

Native and invasive raccoon populations host distinct microbiomes that may facilitate invasion

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Abstract

While microbiome associations are known to impact species survival, the extent to which microbiome variation aids in invasion dynamics is largely unknown. Here, we studied the microbiome diversity and composition of native and invasive raccoon populations to assess the microbiome's role in invasion success. We found that native and invasive populations possess highly distinct microbiomes, with the invasive populations displaying greater gut and oral microbiota diversity without signs of instability or functional decline. Significant differences were found between the microbiota of each pair of invasive populations. The core microbiome of introduced populations consisted of a higher number of taxa than that found in native populations, suggesting a lack of microbiome deterioration in invasive populations. However, this may also reflect insufficient time for the establishment of a stable microbiome. Nevertheless, we found no evidence of reduced diversity of the microbiota linked to the population bottleneck or of increased microbiome dispersion indicating lower community stability. Native populations exhibited no microbiome differentiation between distant locations and showed lower, but still reasonably high, alpha diversity, suggesting the stability of the microbiome. Our findings imply that the invasion process changed the microbiota composition while preserving functional capability across different populations. This study highlights the microbiome as a potential factor aiding the adaptation of invasive populations.

Key words: Invasion, invasive species, microbiome, microbiota, *Procyon lotor*, raccoon

Introduction

Human activities have greatly increased the rates of migration by deliberately or accidentally transporting and introducing large numbers of species to areas beyond natural biogeographic barriers (Perrier et al. 2020). Invasive alien species (IAS), those that become detrimental to recipient ecosystems, are the second most common threat, after habitat loss, associated with species extinctions since AD 1500 (Bellard et al. 2016). Understanding the factors facilitating adaptation of invasive species outside natural ranges is crucial for mitigating their impact. The factors contributing to IAS success include habitat matching (Coulter et al. 2022) or release from native enemies, giving an advantage to IAS over local species (Liu and Stiling 2006). The maintenance of genetic diversity enabling flexibility in adaptation to novel environmental characteristics is also fundamental to the long-term success of invasive species (Kołodziejczyk et al. 2025).

Host–microbiome associations affect host fitness in multiple ways and can be the source of host plasticity (Kolodny and Schulenburg 2020) if that host encounters novel environmental conditions. Indeed, host microbiomes may rapidly increase the host's tolerance to abiotic stress by increasing thermal tolerance (Fontaine et al. 2022), stimulating immune response (Chiu et al. 2017), protecting the host from pathogens (Pickard et al. 2017), or widening dietary niche by enabling metabolism of novel resources (Henry et al. 2021). Given the close relationship between a host and its dependent microbial community, Rosenberg and Zilber-Rosenberg (2018) state that an organism should be viewed as "the holobiont," with the host and its microbiome functioning as a biological entity. Microbes, with larger effective population sizes and shorter generation turnover, may evolve novel functions faster than their hosts (Koonin and Wolf 2012; Ferreiro et al. 2018). Hosts may acquire these adaptive microbes from the local environment, allowing instant adaptation. Nevertheless, the role of the microbiome in wild populations, and even more so in invasive systems, remains largely unexplored (Couch and Epps 2022).

Within a species, microbial communities can vary and have been shown to be influenced by host genotype (Marino et al. 2017), sex (Cong et al. 2016), host infection status (Jani and Briggs 2014), environmental conditions (Wong and Rawls 2012; Aires et al. 2016), and genotype-by-environment interactions (Macke et al. 2017). Variation reflecting specific habitats, including host diet, plays a large role in shaping gut microbial community composition and structure (Eichmiller et al. 2016). Nevertheless, host populations are characterized by a core microbiome that can be defined as a set of microbial taxa specific to a host or environment (Neu et al. 2021). The core taxa are hypothesized to represent ecologically and functionally important microbes. The size and composition of the core microbiome was shown to predict human gut health (Bäckhed et al. 2012) or the responses of organisms to anthropogenic climate change (Hutchins et al. 2019). A large microbiome core should be indicative of high population performance and well-established host–microbiome associations. On the other hand, it has been proposed that the level of dissimilarity in microbiome composition between individuals may indicate a deteriorated host condition. This can be reflected by an increased variance of microbiota (dispersion effects), resulting in higher inter-individual differences due to microbiome instability (Moeller et al. 2013; Lavrinienko et al. 2020).

Invasion processes are predicted to affect the host microbiome (Aires et al. 2016), and the role of the microbiome in invasion processes is increasingly recognized (Romeo et al. 2025). During introduction and range expansion, invasive hosts bring their microbes with them, but a subset of microbial taxa residing in the native host can be lost upon invasion (Kohl 2020; Zepeda-Paulo et al. 2018) in the same way that genetic diversity is stochastically lost when limited numbers of individuals are introduced into a new habitat (Frankham 2005). A shift in microbiome composition between native and invasive ranges has been observed in recent studies (Romeo et al. 2025). Nevertheless, when the microbiome is densely populated and diverse, as in the case of the gut microbiome, strong host–microbiome associations make such a shift less likely (Kohl 2020). The loss of associated microbes, especially those forming the core microbiome, could potentially affect individual fitness. However, invasive species are also exposed to locally available microbiota, creating a changed microbiome over time. In native populations, on the other hand, we might expect less rich but more stable microbial communities, in which novel competitive interactions between microbes

and between microbes and their hosts had sufficiently long time to establish, as reviewed in Ghoul and Mitri (2016).

In contrast to reduced diversity in the invaded range, microbiome diversity may be higher within invasive hosts due to the formation of new relationships with locally available microbes (Himler et al. 2011) or due to a decrease in immune-mediated control of novel microbes (Foster et al. 2017). Higher individual microbiome diversity could potentially increase host performance (Bestion et al. 2017; Stoffel et al. 2020), increasing the chances of invasive species adaptation with the acquisition of beneficial microbes (Martignoni and Kolodny 2024). For example, the phylogenetic diversity of the microbiome of invasive marine herbivorous fishes increased as populations established further from the native range and the local microbiota became increasingly different from the native microbiome (Escalas et al. 2022). The increase was associated with changes in microbiome functions related to the metabolism of short-chain fatty acids and putatively enabled local adaptation (Escalas et al. 2022). Similarly, the microbiome of two invasive hybrid carp species was characterized by high diversity and a high proportion of genes coding for putative enzymes related to their diet (Zhu et al. 2020). During species invasions, an increase in microbiome diversity may help organisms cope with novel environmental conditions or climate changes, potentially from the formation of novel host–microbiome associations. Changes in microbiota may occur due to the loss of specific taxa during introduction, leading to divergent microbiota compositions between native and invasive ranges. Such shifts may not necessarily correlate with a change in the function of the microbiota. The functional redundancy of bacterial taxa (i.e., members of the community with similar functional niches that can substitute for one another) may allow fulfillment of required genomic pathways in both environments (Louca et al. 2018). Although functional redundancy is a known property of the human microbiome (Tian et al. 2020), it has not yet been validated as a factor facilitating species invasions. On the other hand, positive associations between high microbiome diversity and host performance are not unequivocal (Williams et al. 2024), and a number of studies on animal host microbiomes show opposite results (Nelson 2015). For example, high individual microbiome diversity was shown to be associated with poor health in giant pandas (Williams et al. 2016) and chronic laminitis in horses (Steelman et al. 2012). It could therefore be hypothesized that high microbiome diversity of invasive populations may indicate poor performance in the invasive range.

In this study, we analyzed the microbiomes of the raccoon (*Procyon lotor*) from invasive populations in Europe and native populations in North America to test the hologenome hypothesis of invasion. In general, we hypothesized that the gut and oral microbiome of invasive populations would act in concert with the host genome to facilitate invasion success of the species. The raccoon is an omnivorous species that exhibits high plasticity in food choice (Rulison et al. 2012), making it a good model for studying the role of the microbiome. The first successful introduction of raccoons in Europe occurred in Germany in the 1930s with a limited number of individuals (Jernelöv 2017). Unfortunately, information on the invasion source and their microbiome composition is lacking. Genetic analyses of a large set of microsatellite loci and mitochondrial DNA based on whole-country sampling suggest that the current German population of raccoons was established by at least four small-scale, independent introduction events (Fischer et al. 2017) that led to subsequent mixing of genetically divergent invasive populations and

the recreation of relatively high levels of genetic diversity (Biedrzycka et al. 2014). Over time, the invasive range spread eastward, growing into a viable population in western Poland. In contrast, an isolated and stable population of invasive raccoons in the Czech Republic, established in the early 2000s from individuals that escaped from captivity, exhibited lower genetic diversity (Biedrzycka et al. 2014, 2019). In this study, we compare microbiome diversity between invasive European populations of raccoon and two separate populations from the native range (i.e., Florida and Nebraska). These two native populations are demographically stable and occupy similar habitat despite the large geographic distance between them (Jones et al. 1983; Troyer et al. 2014).

To assess the potential role of microbial diversity in the successful spread of raccoons in the invasive range, we characterized the gut and oral microbiome in native and invasive populations. We sought to test three non-mutually exclusive hypotheses concerning microbiome diversity: (i) demographic processes that shaped the genetic diversity of invasive populations will be reflected in microbiome diversity; (ii) the microbiomes of invasive populations will be differentiated from the native ones as a result of microbial taxa loss and gain, but a certain number of core taxa should be maintained, provisioning crucial microbiome functions; (iii) the differential composition of the population microbiome will affect its functional profile, potentially affecting population performance. Overall, this study provides a framework for evaluating how the interaction of the microbiome and host relates to invasion success.

Materials and methods

Sample collection

From each raccoon individual, we collected oral and fecal swabs. We sampled two populations in their native range, near Melbourne, Florida (FL: 25 fecal and 21 oral swab samples), and between Lincoln and Syracuse, Nebraska (NE: 7 fecal and 9 oral swab samples). From the invasive range, samples were collected from a mixed natural and urban habitat surrounding Kostrzyn, Poland (PL: 93 fecal and 73 oral swab samples), in the area between Sonnenberg and Fulda, Germany (GE: 10 fecal and 16 oral swab samples), and around Tovacov and Troubky, Czech Republic (CZ: 35 fecal and 31 oral swab samples) (Fig. 1). Pairs of swabs (fecal and oral) were taken from each individual. Samples from PL were collected between 2021 and 2022, from CZ in 2021 and 2022, from GE in 2022, and from FL and NE in 2023. Samples from Florida were collected from adults transported to the Florida Wildlife Hospital, Inc., during euthanasia or surgical anesthesia directly after a vehicle collision, while samples from Nebraska were collected as a result of local pest control programs. In the invasive range, samples were obtained thanks to game management activity in Poland, Germany, and the Czech Republic. Sex and age class (juvenile or adult) of each individual from PL and CZ were recorded. All samples were collected with sterile, microbiological-grade transport swabs at a depth of 4 cm from the rectum for fecal swabs or inside the mouth at the inner side of the cheek and gums for oral swabs. All fecal and oral swabs were immediately stored in 96% ethanol to minimize contamination from environmental bacteria. The samples were stored at -20 °C prior to DNA extraction.
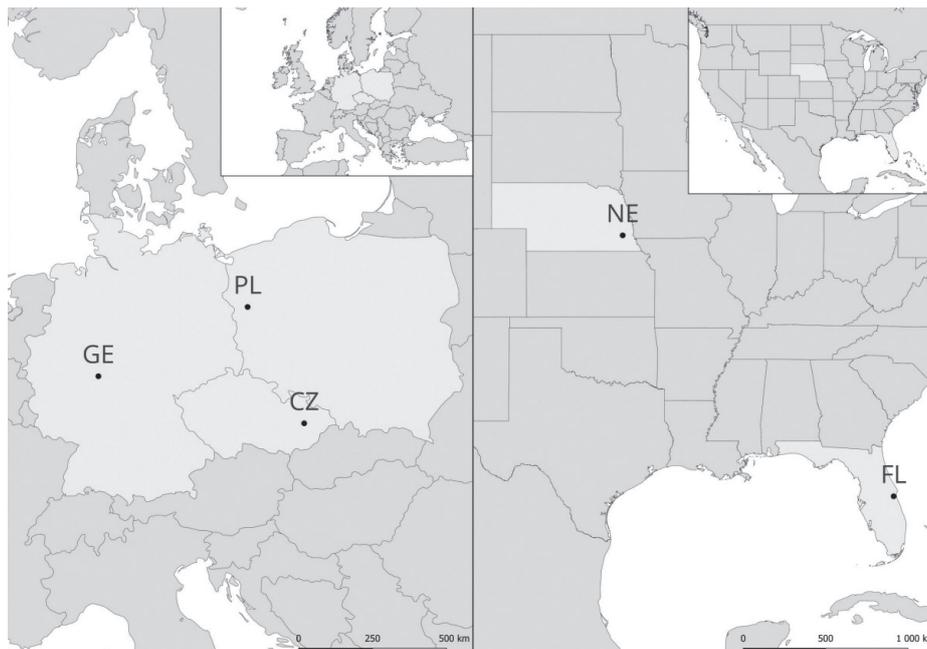
Figure 1. Geographic locations of studied populations. Invasive populations: GE (50.3684, 9.0234), PL (52.5645, 14.7138), and CZ (49.4263, 17.3033) on the left; native populations: FL (28.2839, -80.7770) and NE (40.6602, -96.1825) on the right side of the map.

DNA extraction and genetic metabarcoding library preparation

DNA was extracted from swabs using the NucleoSpin Tissue XS kit for DNA from cells and tissue (Macherey-Nagel) according to the manufacturer's protocol in the pre-PCR dedicated area. One extraction blank was included per extraction batch. DNA was quantified using a Qubit Fluorometer (ThermoFisher Scientific). For assessment of microbiome diversity, PCR was performed with modified indexed bacterial primers 16SF_ins0 (GTGCCAGCMGCCGCGGTAA), 16SF_ins3 (TGAGTGCCAG-CMGCCGCGGTAA), 16SR_ins0 (GGACTACHVGGGTWTCTAATCC), and 16SR_ins3 (TGAGGACTACHVGGGTWTCTAATCC) (Kozich et al. 2013). Indexing enabled the creation of four combinations of primers, which reduced costs. Amplification was performed using HotStart polymerase (Qiagen) under the following conditions: an initial denaturation step of 15 minutes at 95 °C, 32 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 1 minute, with a final extension step at 72 °C for 10 minutes. Each sample was amplified twice, and the resulting products were combined. A blank control was included in each amplification reaction for each primer combination to monitor cross-contamination. PCR products were visually verified via gel electrophoresis on a 1.5% agarose gel and purified using MagSi-DNA beads (magtivio). A second PCR was performed using indexed i5 and i7 primers, enabling two-step indexing. For each plate, we used a combination of eight unique forward and twelve unique reverse primers with 8-base indexes. The protocol was performed as follows: a denaturation step of 15 minutes at 95 °C, 12 cycles of annealing at 95 °C for 30 seconds with HotStart polymerase (Qiagen), 50 °C for 20 seconds, and 72 °C for 20 seconds, followed by a final extension step at 72 °C for 5 minutes. Products were purified, verified, pooled, and quantified using a Qubit Fluorometer. Prepared libraries were sent for sequencing to Novogene (UK) Ltd, using the Illumina NovaSeq 6000 platform in paired-end mode.

Bioinformatic processing

The raw reads had a length of 300 bp and were imported into QIIME2 (version 2024.10; Bolyen et al. 2019), where quality control was performed. Primer removal, denoising, and chimera filtering were performed using the DADA2 plugin within QIIME2. Taxonomic assignment of reads was conducted using a Naïve Bayes classifier trained on the SILVA database (version 138.2) with the q2-feature-classifier plugin. All ASVs (amplicon sequence variants) present in less than 2% of all samples were removed from the dataset. Prior to statistical analysis, sequences classified as eukaryotic, unassigned, or unidentified below the kingdom level were filtered out. Before all analyses, ASV read count data were rarefied to the lowest read count observed in the dataset. The rarefaction threshold was set to 8000. For the rarefaction plot, see Suppl. material 2: fig. S1.

Statistical analysis

Within-population homogeneity tests

Individual microbiome structure and diversity can be affected by host sex (Cong et al. 2016), age (Sadoughi et al. 2022), or season (Maurice et al. 2015). Therefore, we first tested whether the opportunistic mode of sample collection might influence population microbiomes. We performed analyses of alpha and beta diversity using samples from our most well-sampled population, PL. First, we ran analyses separately for fecal and oral samples to test for the effect of 1) sampling year (2021, 2022), 2) sampling season (winter, spring, summer, autumn), 3) sex, and 4) age, to calculate alpha diversity measures using the q2-diversity plugin implemented in QIIME2. We calculated two alpha diversity measures: Shannon's diversity index, which accounts for both the abundance and evenness of the taxa present (Shannon 1948), and Faith's phylogenetic diversity, which incorporates phylogenetic relationships between microbial taxa (Faith 1992). The normality of data distribution was assessed with the Shapiro–Wilk test using the dplyr and ggpubr packages in R (version 4.4.1). For normally distributed data, ANOVA was performed to compare alpha diversity metrics. In cases of a significant ANOVA result, post hoc Tukey HSD (honestly significant difference) multiple comparisons of means were performed. For data with distributions significantly different from normality, transformations such as square root or logarithm were applied. In cases where no transformation normalized the distribution, the Kruskal–Wallis test was performed. Second, we calculated beta diversity measures according to the same grouping using the q2-diversity plugin in QIIME2. We applied Jaccard distance (a qualitative measure of presence–absence taxa between communities; Jaccard 1908), Bray–Curtis distance (a non-phylogenetic quantitative measure accounting for taxa abundance; Bray and Curtis 1957), and unweighted (presence/absence) and weighted (by ASV abundance) UniFrac distances (Lozupone et al. 2006). Differentiation was assessed using PERMANOVA (permutational multivariate analysis of variance, 999 permutations) on Bray–Curtis, Jaccard, unweighted, and weighted UniFrac distances, with the aforementioned variables as predictors. There were no significant differences in alpha diversity measures for either fecal or oral microbiomes between subgroups (see Suppl. material 1), and most beta diversity comparisons were non-significant, with only 17 out of 96 being significant (see Suppl. material 1). Most significant

results (10 out of 96) were obtained for beta diversity comparisons between years (2021 vs. 2022), suggesting differences in composition and/or dispersion of population microbiomes likely driven by temporary environmental conditions. As the majority of samples were collected within a single year (except for PL and CZ), and given the large geographic distances between populations, we did not assume concurrent weather conditions. Therefore, we assumed homogeneity of both fecal and oral microbiomes within populations for all downstream analyses. This allowed us to use the most uniform sample size possible, which is crucial for intra-population comparisons of alpha and beta diversity.

Microbiome differentiation between native and invasive populations and their diversity

Given large sample size discrepancies between sampling sites, we tested whether the two populations from the native region were discrete enough to require separation for future analyses or whether they could be combined into a single population representative of the native distribution. We tested whether within-population microbiome diversity measures (alpha diversity) and between-population measures (beta diversity) performed between native populations showed significant differentiation (see within-population homogeneity tests for methods). Indeed, NE and FL populations did not exhibit differentiation for any indices (see Suppl. material 2: tables S1, S2). We therefore combined samples from these two populations into a single native population, USA (32 fecal and 30 oral samples), for subsequent analyses. To further equalize sample sizes, for all downstream analyses, we randomly subsampled the PL population to 35 and 31 samples for fecal and oral samples, respectively.

To test whether within-population microbiome diversity reflects genetic diversity levels of studied populations, we calculated alpha diversity measures (Shannon's diversity index and Faith's phylogenetic diversity) as described above and visualized results in the ggplot package.

To test whether microbiome differentiation between populations reflects genetic differentiation between them, we calculated beta diversity using Jaccard, Bray–Curtis, unweighted, and weighted UniFrac distances. We performed pairwise PERMDISP (tests of homogeneity of multivariate dispersion among groups) in QIIME2. A significant result indicates that groups differ in the level of variation (Bakker 2024). Higher variability in dispersion of beta diversity distances across populations is hypothesized to be an indicator of lower community stability and could reflect the loss of certain microbiome taxa during population establishment in the invasive range. Further, we tested differences in beta diversity distances using PERMANOVA. Differentiation between samples was visualized using PCoA in the ggplot package in R (version 4.4.1).

Microbiome composition and functional profile

To describe the composition of gut and oral microbiomes in native and invasive populations and assess whether it reflects invasion processes in terms of the number of enriched and depleted taxa, we tested for differential abundance of bacterial ASVs between populations and identified taxa contributing most to inter-population microbiome diversity using ANCOM-BC (analysis of compositions of microbiomes with bias correction; Lin and Peddada 2020) in QIIME2. Analy-

ses were performed on three populations (PL, CZ, and USA), identified bacteria at the family or order level, and used a significance threshold of 0.05. The GE population was excluded because small sample sizes and unequal sample sizes between populations can lead to false positives (Lin and Peddada 2020). The core microbiota (i.e., microbial taxa present across multiple samples within a habitat; Neu et al. 2021) were identified using the phylogeny-based algorithm PhyloCore (Ren and Wu 2016) with a prevalence threshold of 0.9. Due to the use of short 16S fragments and limited availability of marker sequences in microbial reference databases, interpretations below the genus level were considered unreliable. The frequency of core microbiome taxa relative to all taxa present in individual samples was then used to calculate core density. We set the threshold at 0.6 (60% of taxa in the individual belonging to the core microbiome). Estimating whether individuals exhibit a dense core versus a sparse core is necessary to evaluate microbiome stability (Björk et al. 2018). The nearest-sequenced taxon index was calculated for each sequence with Picrust2 (Douglas et al. 2020) (for results, see Suppl. material 3). Most results, however, were below 2, the threshold considered limiting for confident interpretation of taxa.

To determine functional profiles of microbiome communities and assess whether potential differences in gut and oral microbiome composition translate to differences in function, we used Tax4Fun2 software (Wemheuer et al. 2020). We applied the reference database where 16S rRNA gene sequences were clustered at 99% similarity (99NR). The minimum threshold of similarity for ASVs was set at 97%. Functional predictions were generated by assigning ASVs to KEGG Orthology pathways (Wemheuer et al. 2020). Data were analyzed and visualized using microeco, seqinr, and file2meco packages following the microeco (v1.10.0) tutorial, and PERMANOVA was performed using the vegan package in R (version 4.4.1) to assess functional microbiome differences between populations. Because of short read length and limited reference sequences, we could not resolve taxa to the species level, which restricted functional conclusions.

Results

We analyzed a total of 112 fecal swabs and 108 oral swabs from four populations: invasive Polish, Czech, and German, and native USA. The mean raw sequencing depth was 110,649 for fecal samples and 137,475 for oral samples. After filtering, the mean number of reads of ASVs per sample was 68,970 (SD = 50,786) for fecal samples and 80,731 (SD = 58,321) for oral samples. The mean percentage of merged reads was 84% for fecal samples and 76% for oral samples. The total number of reads for fecal samples was 12,966,399 and for oral samples 14,047,213.

Microbiome differentiation between native and invasive populations and their diversity

Although estimates of alpha diversity may be underestimated for GE due to smaller sample size compared to other populations (Willis 2019), we decided to include these results, as the GE population represents the raccoon invasion core and primary site of introduction. Nevertheless, alpha diversity measures for this population should be interpreted with caution. There was a significant difference in alpha diversity in the fecal microbiome between populations for both Shannon's
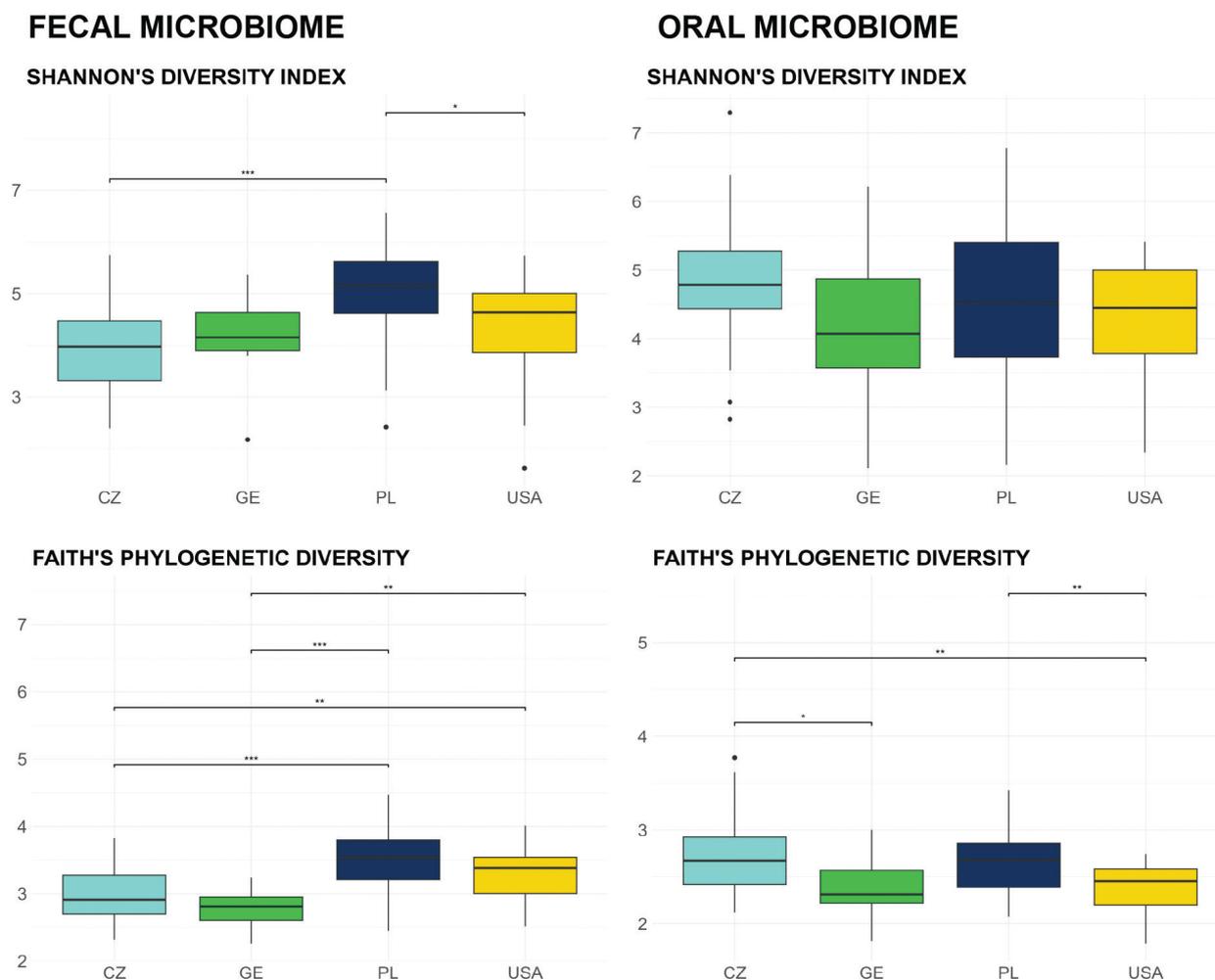
Figure 2. Alpha diversity measures for native and invasive population microbiomes. Measures for fecal microbiomes are displayed in left-side plots, whereas measures for oral microbiomes are displayed in right-side plots. Significant differences were detected between PL–CZ and USA–PL (Shannon's DI for fecal microbiome), between PL–CZ, USA–CZ, PL–GE, and USA–GE (Faith's PD for fecal microbiome), and between GE–CZ, USA–CZ, and USA–PL (Faith's PD for oral microbiome) (Suppl. material 2: table S4).

DI (F = 6.659; p = 0.000368) and Faith's PD (F = 11.4; p = 1.57^{e-06}) and in the oral microbiome regarding Faith's PD (F = 6.984; p = 0.000255) (see Suppl. material 2: table S3). The alpha diversity of the fecal microbiome was significantly higher in PL than in CZ, USA, and GE. However, the alpha diversity of the USA was significantly higher than that of CZ and GE. The alpha diversity of the oral microbiome was significantly higher in CZ than in GE or the USA. However, PL showed significantly higher alpha diversity than the USA (Fig. 2; Suppl. material 2: table S4).

The only significant difference detected by PERMDISP was between the fecal microbiome of GE and USA on unweighted UniFrac distance and between the oral microbiome of CZ–PL and CZ–USA on weighted UniFrac (Suppl. material 2: table S5). PERMANOVA results of diversity measures for these pairs should be approached with caution, as observed differences may be influenced by dispersion rather than shifts in composition. The lack of significant PERMDISP differences for other pairs indicated that differences in composition were not explained by within-group variance. PERMANOVA on unweighted UniFrac detected significant differentiation in both fecal and oral microbiomes between all population

Table 1. Results of beta diversity metrics. Pairwise PERMANOVA results for Jaccard, Bray–Curtis, unweighted, and weighted UniFrac distances on fecal (left) and oral (right) microbiomes. The number of permutations was set to 999; all *p*-values were adjusted with the Benjamini–Hochberg procedure. Asterisks indicate significant results.

	Group 1	Group 2	FECAL				ORAL			
			Sample size	F	R²	p	Sample size	F	R²	p
Jaccard distance	CZ	GE	44	1.65	0.0564	0.004*	46	1.906	0.048	0.001*
		PL	67	2.544	0.0447	0.002*	61	2.2	0.036	0.001*
		USA	66	3.325	0.0568	0.002*	59	3.118	0.057	0.001*
	GE	PL	43	1.454	0.0331	0.02*	47	2.129	0.046	0.001*
		USA	42	1.857	0.043	0.004*	45	2.408	0.053	0.001*
	PL	USA	65	2.82	0.0433	0.002*	60	3.136	0.051	0.001*
Bray-Curtis distance	CZ	GE	44	2.444	0.074	0.004*	46	1.981	0.055	0.009*
		PL	67	4.537	0.061	0.002*	61	2.342	0.036	0.001*
		USA	66	5.43	0.084	0.002*	59	2.941	0.051	0.001*
	GE	PL	43	1.298	0.031	0.142	47	2.388	0.046	0.001*
		USA	42	2.159	0.051	0.006*	45	2.53	0.055	0.001*
	PL	USA	65	3.243	0.047	0.002*	60	2.509	0.040	0.002*
Unweighted UniFrac distance	CZ	GE	44	2.484	0.3407	0.004*	46	2.401	0.055	0.002*
		PL	67	4.441	0.581	0.001*	61	2.431	0.054	0.002*
		USA	66	8.081	1.244	0.001*	59	11.194	0.155	0.002*
	GE	PL	43	2.925	0.373	0.001*	47	2.000	0.036	0.002*
		USA	42	4.326	0.683	0.001*	45	5.761	0.120	0.002*
	PL	USA	65	7.161	1.241	0.001*	60	9.245	0.127	0.002*
Weighted UniFrac distance	CZ	GE	44	0.815	0.023	0.544	46	0.822	0.029	0.509
		PL	67	3.327	0.108	0.019*	61	2.685	0.064	0.033*
		USA	66	6.233	0.125	0.003*	59	7.794	0.123	0.003*
	GE	PL	43	1.349	0.058	0.256	47	1.238	0.035	0.313
		USA	42	3.874	0.088	0.019*	45	4.405	0.093	0.01*

pairs, whereas weighted UniFrac distances differed significantly in both fecal and oral microbiomes between CZ–PL, CZ–USA, GE–USA, and PL–USA (Table 1), indicating differences in the most abundant taxa. PERMANOVA results on Jaccard distance were significant in all cases for both fecal and oral microbiomes. Differences in Bray–Curtis distance were significant in all pairwise comparisons for both fecal and oral microbiomes, except for the GE–PL pair in the fecal microbiome (Table 1; Fig. 3). In general, differentiation expressed as unweighted UniFrac distance was higher than that detected by Jaccard distance, indicating that differentiation was driven by more phylogenetically distant taxa (Table 1). Non-significant between-population estimates for Bray–Curtis distances (GE–PL for fecal) and weighted UniFrac distances (GE–PL for fecal; CZ–GE and GE–PL for oral microbiome) indicate that differences were caused by presence/absence of taxa rather than their abundance. For all distance measures, the largest separation was observed between the native and all invasive populations (Table 1).

Microbiome composition and functional profile

The results of the ANCOM-BC differential abundance test (Fig. 4) indicated that more taxa were enriched in PL compared to the USA (in the fecal microbiome, seven taxa; in the oral microbiome, 28 taxa) than depleted (in the fecal microbiome,
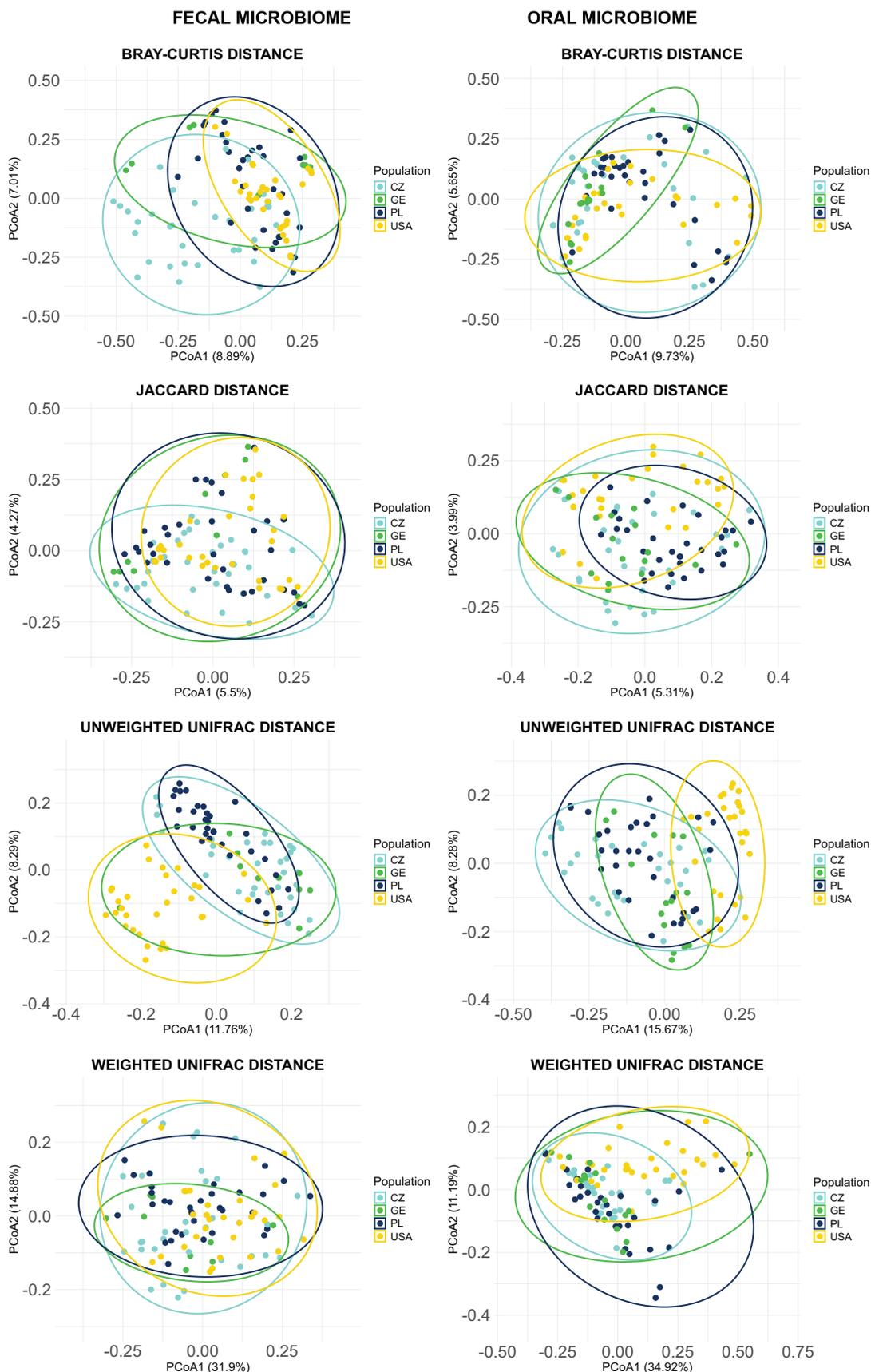
Figure 3. Beta diversity measures for native and invasive population microbiomes. Measures for fecal microbiomes are displayed in left-side plots, whereas measures for oral microbiomes are displayed in right-side plots. The significance of inter-population differences in diversity is given in Table 1.
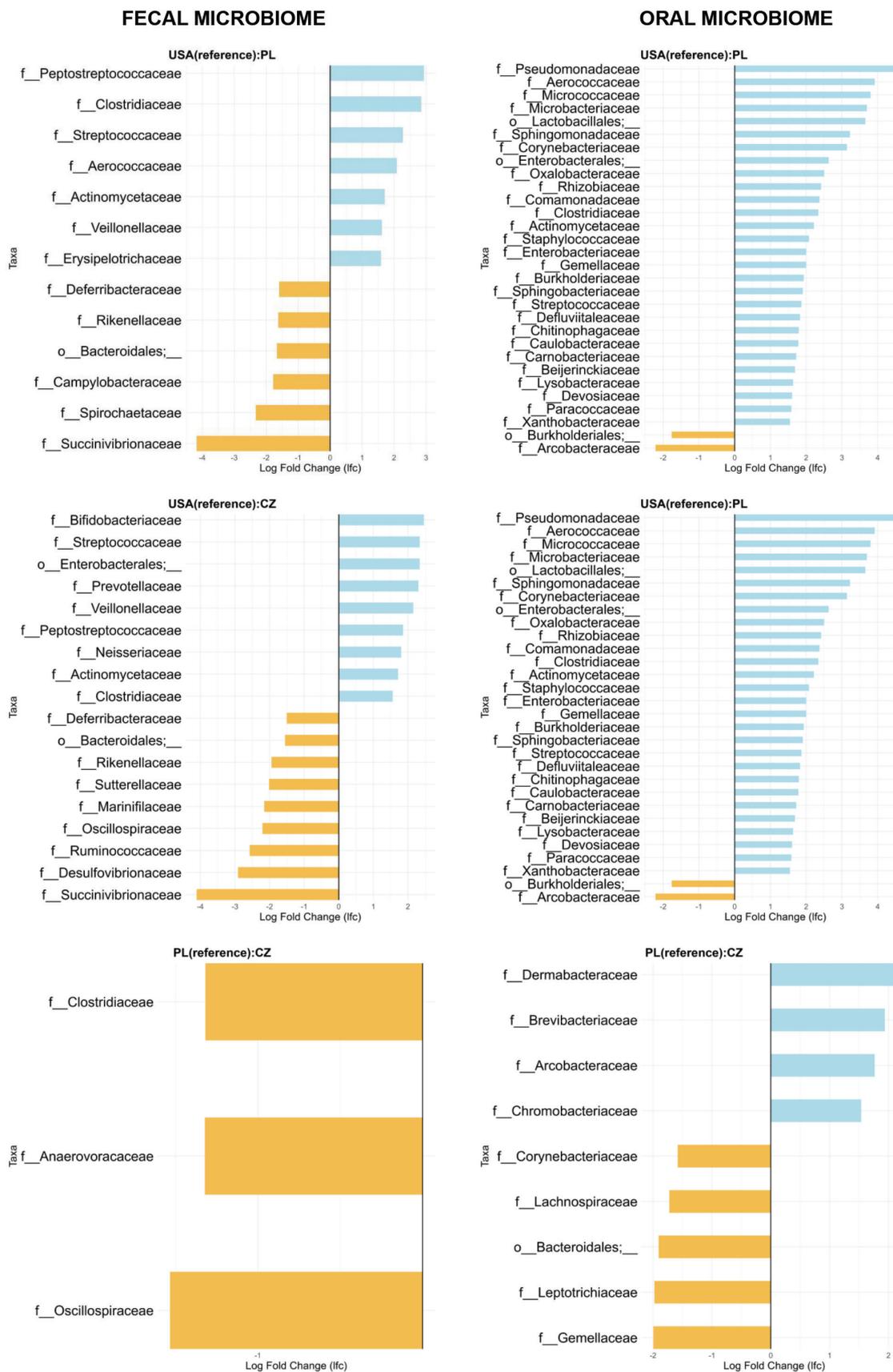
Figure 4. Differential abundance of taxa. Plots display differential abundance of significantly different taxa between populations from fecal (left) and oral (right) microbiomes detected by ANCOM-BC; o – order, f – family. Full taxonomy is shown in Suppl. material 2: tables S6, S7.

93

six taxa; in the oral microbiome, two taxa). The fecal microbiome exhibited nine enriched taxa and nine depleted taxa when comparing CZ to the USA (Fig. 4). However, comparison of the oral microbiome found that 20 taxa were enriched in CZ and only seven depleted compared to the USA. PL and CZ differed significantly in the abundance of one family, Oscillospiraceae, which was depleted in the CZ fecal microbiome. In the oral microbiome, abundance differed in nine taxa: four were enriched and five depleted in CZ compared to PL (Fig. 4).

In the fecal microbiome, the highest number of core components was detected in GE (22), followed by PL (17) and CZ (14), with the lowest number in USA (13). In the oral microbiome, we detected the highest number of core components in PL (34), followed by CZ (31), GE (17), and, as with fecal samples, the lowest number in USA (16). Six taxa in the fecal microbiome and five taxa in the oral microbiome were shared between all studied populations. Five taxa in the fecal microbiome as well as five taxa in the oral microbiome were present only in the native population. Core taxa overlapping among all invasive populations (excluding native taxa) were represented by four fecal and six oral microbiome taxa (Fig. 5). In the fecal core microbiome of all populations, we detected *Clostridium*, Lachnospiraceae, *Bacteroides*, *Helicobacter*, *Fusobacterium*, and *Escherichia/Shigella*. Taxa assigned to the fecal core microbiome of all invasive populations but absent in native populations were Actinobacteria, *Streptococcus*, Prevotellaceae, and Moraxellaceae. Lactobacillales, Ruminococcaceae, Peptostreptococcales-Tissierellales, *Cetobacterium*, and Sutterellaceae were components only of the native fecal core microbiome. We identified *Streptococcus*, Lachnospiraceae, *Fusobacterium*, *Glaesserella*, and *Moraxella* in the oral core microbiome of all populations. Components of the oral core microbiome of all invasive populations were *Bacteroides*, *Helicobacter*, *Burkholderia-Caballeronia-Paraburkholderia*, *Escherichia/Shigella*, Lysobacteraceae, and *Pseudomonas*. The native oral core microbiome consisted of Actinobacteria, Mycoplasmataceae, Campylobacterales, Leptotrichiaceae, and Neisseriaceae.
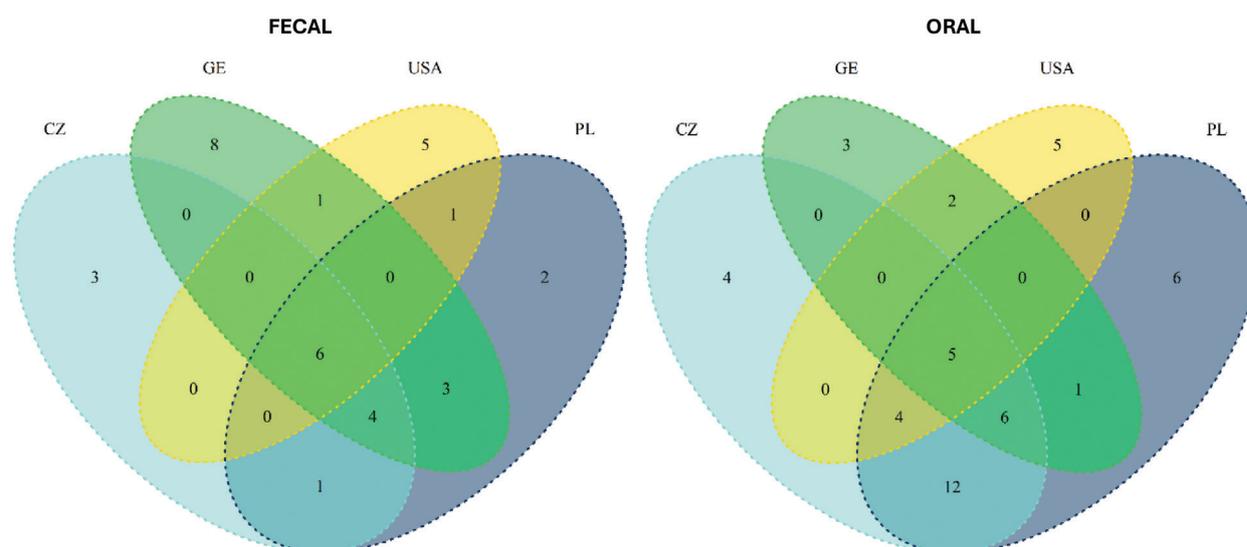

Figure 5. Core microbiota by population in fecal and oral microbiomes, taking into account ecological coherence of higher-rank taxa and calculating core taxa at different taxonomic levels. The number of core microbiota attributed to each studied population is shown for fecal (left) and oral (right) microbiomes. Core microbiome taxa are listed in Suppl. material 2: tables S8, S9. The most common taxa in the fecal core microbiome were *Bacteroides*, *Fusobacterium*, and *Helicobacter*, whereas in the oral core microbiome they were *Glaesserella*, *Moraxella*, and *Porphyromonas*.
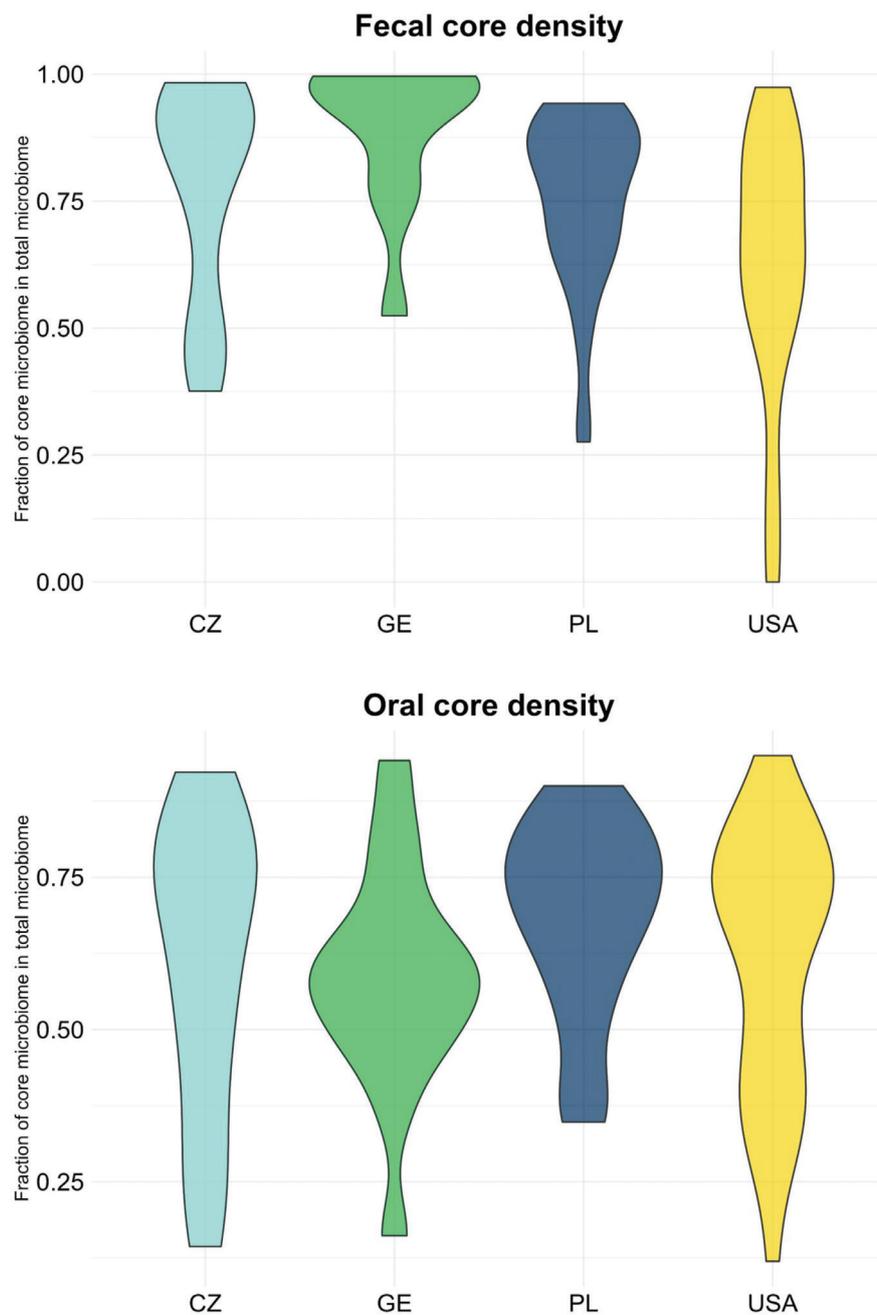
Figure 6. The core density in populations. The density of fecal (top) and oral (bottom) core microbiomes. The frequency of the core microbiome within the total microbiome composition is shown for individuals from four studied populations.

Core density was calculated as the frequency of taxa belonging to the core microbiome present in each individual from that specific population (Fig. 6). In the native population, a core density ≥0.6 was detected in most individuals (20 out of 32 in the fecal microbiome and 18 out of 29 in the oral microbiome). Among invasive populations, the number of individuals with core density ≥0.6 was even higher: in PL, 31 out of 35 individuals in the fecal microbiome and 23 out of 31 in the oral microbiome; in CZ, 26 out of 35 (fecal) and 17 out of 31 (oral); and in GE, 9 out of 10 (fecal) and 6 out of 16 (oral).

The functional microbiome analysis revealed that over 70% of pathways present in each population were related to metabolism (Suppl. material 2: figs S3, S4). At a more detailed level, pathways linked to global and overview maps, carbohydrate metabolism, amino acid metabolism, and membrane transport were most frequently represented (Suppl. material 2: figs S3, S4). PERMANOVA revealed marginally significant differences in pathway predictions between PL–GE, PL–USA, and GE–USA in the fecal microbiome and between CZ–USA and PL–USA in the oral microbiome (Suppl. material 2: table S10).

Discussion

In this study we used raccoons as a model system to investigate microbiome diversity and composition in native and invasive populations. We sampled populations of raccoons from throughout Europe, where raccoons first invaded approximately 90 years ago, and across two populations from the native range. Raccoons are known to use a wide range of food resources (Bartoszewicz et al. 2008; Rulison et al. 2012), invading a variety of habitats, and should putatively rely heavily on microbial services. We found that native and invasive raccoon populations had highly distinct microbiotas. We detected a significant shift in microbiome composition between native and invasive populations, putatively related to different environmental and dietary niches, but possibly also related to loss and gain of microbial taxa during species invasion. In the native range, we identified gut and oral microbiomes that exhibited less diversity than in Europe and little differentiation among populations, despite the large geographic distance between them. This could suggest that native raccoons have developed strong and stable host–microbiome associations related to their diet, relying on human-related food, and putatively contributing to their success in urban habitats. We found that invasive populations were characterized by higher population microbiome diversity, and the core microbiomes in introduced populations consisted of more taxa than those found in native populations. Despite differences in microbiome composition between native and invasive populations and among invasive populations, we did not detect differences at the functional level. Our results indicate that the raccoon microbiome, both in the native and invasive ranges, may facilitate adjustment to local conditions, although through different types of host–microbiome associations. Below we address each of the three hypotheses tested in this study and how our results provide evidence that invaders can form new microbial associations, which may play a role in invasion success, putatively allowing microbially mediated adaptation to invasive habitats.

Microbiome diversity mirrors invasion pathways

We first hypothesized that processes associated with specific demographic events that shaped the genetic diversity of invasive populations would also be reflected in microbiome diversity. Our findings supported this hypothesis in that we did not detect any apparent signs of decreased microbiota alpha diversity in invasive populations that could be attributed to an invasion-related population bottleneck. Although patterns of microbiome diversity resemble those represented by population genetic diversity (Biedrzycka et al. 2014, 2019; Konopiński et al. 2023), this result does not prove a direct relationship between microbial and specific genetic variants.

Rather, our results show the impact of demographic processes that affected both hosts and their microbiomes. Indeed, the highest alpha diversity of the fecal microbiome was detected in the invasive PL population. Recent studies have shown that spatial proximity of hosts can play an important role in creating microbial diversity by mediating exposure to similar microbes and allowing bacterial taxa transfer between individuals (Phillips et al. 2012; Couch et al. 2020). This could explain the high alpha diversity of the dense PL population compared to the two other invasive populations. Despite originating from the invasion core in Germany around the 1980s (Okarma et al. 2012; Biedrzycka et al. 2014) and being located at the invasion front (Biedrzycka et al. 2020), the PL population is characterized by very high densities (Bartoszewicz et al. 2008; Jernelöv 2017), promoting more intensive social interactions between raccoons (Fisher et al. 2021). In contrast, the CZ population, which exhibited significantly lower alpha diversity than PL, was established about 20 years later (Anděra and Gaisler 2012) by individuals that escaped from captivity, with no signs of intensive expansion or exchange of individuals with other raccoon populations (Konopiński et al. 2023). At the same time, this population had lower genetic diversity (Biedrzycka et al. 2014, 2019). Positive associations between genetic and microbiome diversity have been found in other species such as invasive tunicates (Casso et al. 2020; Goddard-Dwyer et al. 2021) and marine sponges (Marino et al. 2017). Our study is the first to suggest such a pattern in an invasive mammal. However, more studies, including direct comparisons of individual microbiome and genetic diversity, are needed to evaluate this association in invasive species.

Second, we hypothesized that the microbiomes of invasive populations would be differentiated from the native ones as a result of microbe taxa loss and gain. Our results not only indicated a shift in bacterial communities in invasive raccoon populations compared to those from the native range but also revealed significant differences between invasive populations. The results for the oral microbiome closely mirrored those obtained for fecal samples. The most pronounced difference was due to the occurrence of different taxa rather than differential abundance. Because alpha diversity is relatively high throughout the invasive range, differentiation between invasive populations is likely due to the acquisition of local microbial taxa picked up during their expansion route. Similar patterns were found in invasive signal crayfish, where environmental influences impacted microbiome diversity (Dragičević et al. 2021). The differences between invasive and native raccoon populations suggest a shift in the most abundant microbial taxa, indicating a potential dietary change. This shift could be explained by raccoons in their native range consuming more urban waste, reflecting proximity to human settlements. In contrast, invasive populations tend to inhabit a broader range of environments, including forests and wetlands, where their diet is likely more natural and less influenced by human activity. Differences in microbiome composition can also be related to differences in host genetic background. Although we cannot directly compare individual genetic and microbial differentiation, the genetic structure of native and invasive populations – where establishment from genetically divergent populations remains visible despite intensive gene flow between invasive populations (Biedrzycka et al. 2014) – is well mirrored by their microbiome differentiation. The greatest differentiation, both from other invasive populations and from the native range, was observed in comparisons with CZ, reflecting its different invasion pathway and isolation. This variation was evident in both genetic (Biedrzycka et al. 2014) and microbiome between-population diversity (Fig. 2).

Structural and functional differences between native and invasive microbiomes

The identification and study of core microbial symbionts are of particular interest due to the ecological relevance and potential functionality of microbial taxa that consistently occur in specific habitats, but also because they can be used to identify the health status of individuals (Shade and Handelsman 2012; Astudillo-García et al. 2017). The size of an individual's core microbiome (dense core or sparse core) is indicative of microbiome stability and resilience (Björk et al. 2018). Here we found that in all invasive populations, fecal core microbiome density was shifted towards higher values compared to the combined native population, suggesting a high level of stability in the invasive range (Fig. 6). Invasive populations were also enriched in more taxa than the native population (Fig. 4). Moreover, this difference was even stronger for the oral microbiome, where the core was generally sparser than in the fecal microbiome but enriched in even more taxa relative to the native population (Fig. 4). This enrichment probably reflects a more diverse diet in the invasive range, as suggested by studies showing invasive raccoons to feed on diverse food resources available at any given moment (Michler 2018), whereas native populations are more dependent on continuously available human-related food (Bozek et al. 2007). A positive relationship between diet diversity and gut microbiome diversity has been demonstrated in highly mobile hyenas (Theis et al. 2012) and fur seals (Grosser et al. 2019). Additionally, a high number of individuals possessing a large and dense core microbiome may reflect redundancy of core taxa. Phylogenetically diverse microbial groups present in invasive raccoon microbiomes suggest microbiome resilience. Interestingly, the GE population, represented by a smaller sample size than other populations, exhibited lower alpha diversity but had similar density (90%) and the most diverse fecal core microbiome (22 taxa). This may be related to its early establishment and core location, with substantial exchange of individuals from surrounding regions (Fischer et al. 2015). A delay in the acquisition of local microbes has been proposed as an explanation for the lag phase of invasive species, where the time needed for population establishment is tied to the formation of more diverse microbiota (Martignoni and Kolodny 2024).

We further hypothesized that a shift in microbiome composition associated with raccoon invasion to Europe would be accompanied by impaired microbiome function. Analysis of gene pathways associated with microbial communities displayed weak but significant differences in functional capabilities between populations. The majority of pathways were related to metabolism, mainly global and overview maps and carbohydrate and amino acid metabolism, further confirming the role of gut microbial taxa in nutrient decomposition (Carthew 2021). The minor differences in gene pathway abundance (Suppl. material 2: figs S3, S4) visualized between populations may reflect raccoon plasticity in adapting to different environmental conditions via microbiome composition. Nevertheless, this relatively low level of differentiation is putatively associated with functional redundancy (Louca et al. 2018) of taxa within unique microbiomes, allowing similar functional capabilities across communities. Microorganisms with overlapping roles may compensate for the loss of a beneficial strain by replacing it in metabolic pathways (Youngblut et al. 2019).

We also did not observe increased microbiota community dispersion in invasive populations, which is hypothesized to indicate lower community stability (Lavrinienko et al. 2020). Various types of environmental stress can increase variance in microbiota

composition (dispersion effects), resulting in higher inter-individual microbiome differences (Wu et al. 2016; Halfvarson et al. 2017). Our analyses showed that although studied populations differed in microbiome composition, dispersion of identified taxa was relatively uniform, indicating that novel environmental conditions in the invasive range do not pose challenges that would be reflected in dispersion levels. The only significant differences – between CZ and the native population, and between CZ and PL – are putatively related to differences in diet composition (McFall-Ngai et al. 2013).

Specific microbial taxa

Finally, we hypothesized that despite stochastic shifts in microbiome composition or differences related to specific habitats, the core microbiome taxa would be shared between all populations (native and invasive). The core taxa should represent groups important to host biology and therefore be maintained by host–microbiome coevolution despite stochastic changes induced by introduction to new habitats (Shade and Handelsman 2012; Neu et al. 2021). Due to the lack of species-level taxonomic assignments, we could not draw conclusions about specific functions of particular taxa.

In the core fecal microbiome, we detected six taxonomic groups shared across all studied populations and five groups shared among all invasive populations. A similar number of taxonomic groups were shared for the oral core microbiome, although the specific taxa differed. While we cannot definitively determine the functions of the common taxa, some level of congruence suggests that specific groups are maintained despite changes associated with the invasion process. The detection of numerous, but not necessarily overlapping, bacterial taxa in both native and invasive raccoon microbiomes further supports the finding that raccoon populations from both ranges were able to adapt to their respective habitats despite invasion-related shifts in microbiome composition and differences in population-level microbiome diversity.

The characteristics of the microbiome of native populations

The lack of microbiome differentiation between two distant native populations was surprising. This result could, to some extent, be explained by the relatively low sample size of the Nebraska population, which could affect both the Shannon diversity index (Shannon 1948) and PERMANOVA results (Anderson 2014). However, we did not detect lower alpha diversity in the NE population. Further, the spatial analysis of individual microbiome diversity from the two native populations did not suggest differentiation in composition (Bray–Curtis distance, Suppl. material 2: fig. S2). Although this result must be treated with caution, the lack of differentiation, coupled with lower alpha diversity than in invasive populations, may suggest a highly stable and resistant microbiome. Homogeneity of gut microbiomes has been demonstrated for geographically distinct human populations, suggesting high stability (Jackson et al. 2018). In our study, relatively lower microbiome diversity in the native range may indicate adjustment to local conditions, even if associated with a drop in some diversity measures. The omnivorous diet of raccoons – dependent on current food availability and typically including mollusks, water-related invertebrates and plants, and human-related waste – is typical in both native and invasive populations (Rulison et al. 2012). Nevertheless, the native populations sampled

here are closely associated with urban habitats, which may shift their diet towards human-related food. It is known that such simplified diets lead to low microbiome diversity and can eliminate inter-population differentiation. For example, studies on American black bears revealed that consumption of human-provisioned foods was associated with reduced gut microbial diversity (Gillman et al. 2022). Similarly, in Tome's spiny rats, anthropogenic disturbances led to decreased alpha diversity and shifted beta diversity in gut microbiomes (Fackelmann et al. 2021). On the other hand, it was suggested that the ability to exploit novel food resources in new habitats – as reflected by higher alpha diversity in the invasive PL population – likely played a vital role in the recent successful expansion of raccoons (Rulison et al. 2012). This may suggest that, in contrast to native populations, invasive raccoon populations rely on greater food diversity. Nevertheless, more studies of native raccoon microbiomes are needed to confirm this assumption.

Conclusion

There is an established link between gut microbiome composition and host fitness (Gould et al. 2018). However, knowledge of how the establishment of a species in a new range affects its microbiome, and whether the microbiome plays an important role in invasion success, is limited (but see Minard et al. 2015; Casso et al. 2020; Bankers et al. 2021; Goddard-Dwyer et al. 2021; Escalas et al. 2022). Here we found that the invasion process changes the composition of microbiota in an extremely successful invader, the raccoon. Raccoons possess a high level of genomic diversity created through mixing of divergent invasive populations, allowing for rapid adaptation to novel environments from standing genetic variation (Biedrzycka et al. 2014; Konopiński et al. 2023). Together with high reproductive potential (Ritke 1990; Asano et al. 2003) and opportunistic use of food resources (Michler 2018), these characteristics make the raccoon an extremely successful invader. We revealed that raccoons also possess highly diverse gut microbiota, with no obvious signs of instability and with functional capacity not strongly deviating from that of native populations. Our results suggest that the microbiome reflects the diverse diet of invasive raccoons and, along with relatively high levels of genetic variation in neutral (Uller and Leimu 2011) and adaptive regions of the genome (Day 2015), represents another factor facilitating the successful performance and spread of this invasive species.

Acknowledgments

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.

Funding

Author contributions

Conceptualization: AB. Data curation: JK. Formal analysis: JK. Funding acquisition: AB. Investigation: AB, EH, JK. Methodology: AB, JK. Project administration: AB. Resources: AB, EH. Software: JK. Supervision: AB. Visualization: JK. Writing – original draft: JK, AB. Writing – review and editing: EH.

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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 amplicon DNA sequences have been deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under BioProject accession no. PRJNA1249228.

References

Aires T, Serrão EA, Engelen AH (2016) Host and environmental specificity in bacterial communities associated to two highly invasive marine species (Genus *Asparagopsis*). Frontiers in Microbiology 7: 559. https://doi.org/10.3389/fmicb.2016.00559

Anděra M, Gaisler J (2012) Savci České republiky: Popis, rozšíření, ekologie, ochrana. Academia (Caracas).

Anderson MJ (2014) Permutational multivariate analysis of variance (PERMANOVA). Wiley statsref: statistics reference online, 1–15. https://doi.org/10.1002/9781118445112.stat07841

Asano M, Matoba Y, Ikeda T, Suzuki M, Asakawa M, Ohtaishi N (2003) Reproductive characteristics of the feral raccoon (*Procyon lotor*) in Hokkaido, Japan. The Journal of Veterinary Medical Science 65: 369–373. https://doi.org/10.1292/jvms.65.369

Astudillo-García C, Bell JJ, Webster NS, Glasl B, Jompa J, Montoya JM, Taylor MW (2017) Evaluating the core microbiota in complex communities: A systematic investigation. Environmental Microbiology 19: 1450–1462. https://doi.org/10.1111/1462-2920.13647

Bäckhed F, Fraser CM, Ringel Y, Sanders ME, Sartor RB, Sherman PM, Versalovic J, Young V, Finlay BB (2012) Defining a healthy human gut microbiome: Current concepts, future directions, and clinical applications. Cell Host & Microbe 12: 611–622. https://doi.org/10.1016/j.chom.2012.10.012

Bakker JD (2024) PERMDISP. Applied Multivariate Statistics. https://uw.pressbooks.pub/appliedmultivariatestatistics/chapter/permdisp/

Bankers L, Dahan D, Neiman M, Adrian-Tucci C, Frost C, Hurst GDD, King KC (2021) Invasive freshwater snails form novel microbial relationships. Evolutionary Applications 14: 770–780. https://doi.org/10.1111/eva.13158

Bartoszewicz M, Okarma H, Zalewski A, Szczęsna J (2008) Ecology of the raccoon (*Procyon lotor*) from Western Poland. Annales Zoologici Fennici 45: 291–298. https://doi.org/10.5735/086.045.0409

Bellard C, Cassey P, Blackburn TM (2016) Alien species as a driver of recent extinctions. Biology Letters 12: 20150623. https://doi.org/10.1098/rsbl.2015.0623

Bestion E, Jacob S, Zinger L, Di Gesu L, Richard M, White J, Cote J (2017) Climate warming reduces gut microbiota diversity in a vertebrate ectotherm. Nature Ecology & Evolution 1: 1–3. https://doi.org/10.1038/s41559-017-0161

Biedrzycka A, Zalewski A, Bartoszewicz M, Okarma H, Jędrzejewska E (2014) The genetic structure of raccoon introduced in Central Europe reflects multiple invasion pathways. Biological Invasions 16: 1611–1625. https://doi.org/10.1007/s10530-013-0595-8

Biedrzycka A, Konopinski MK, Hoffman EA, Trujillo AL, Zalewski A (2019) Comparing raccoon MHC diversity in native and introduced ranges: Evidence for the importance of functional immune diversity for adaptation and survival in novel environments. Evolutionary Applications; Advance online publication. https://doi.org/10.1111/eva.12898

Biedrzycka A, Konopiński M, Hoffman E, Trujillo A, Zalewski A (2020) Comparing raccoon major histocompatibility complex diversity in native and introduced ranges: Evidence for the importance of functional immune diversity for adaptation and survival in novel environments. Evolutionary Applications 13: 752–767. https://doi.org/10.1111/eva.12898

Björk JR, O'Hara RB, Ribes M, Coma R, Montoya JM (2018) The dynamic core microbiome: Structure, dynamics and stability. BioRxiv 137885. https://doi.org/10.1101/137885

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, et al. (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37: 852–857. https://doi.org/10.1038/s41587-019-0209-9

Bozek CK, Prange S, Gehrt SD (2007) The influence of anthropogenic resources on multi-scale habitat selection by raccoons. Urban Ecosystems 10: 413–425. https://doi.org/10.1007/s11252-007-0033-8

Bray JR, Curtis JT (1957) An ordination of the upland forest communities of Southern Wisconsin. Ecological Monographs 27: 326–349. https://doi.org/10.2307/1942268

Carthew RW (2021) Gene regulation and cellular metabolism: An essential partnership. Trends in Genetics : TIG 37: 389–400. https://doi.org/10.1016/j.tig.2020.09.018

Casso M, Turon M, Marco N, Pascual M, Turon X (2020) The microbiome of the worldwide invasive ascidian *Didemnum vexillum*. Frontiers in Marine Science 7: 201. https://doi.org/10.3389/fmars.2020.00201

Chiu L, Bazin T, Truchetet M-E, Schaeverbeke T, Delhaes L, Pradeu T (2017) Protective microbiota: From localized to long-reaching co-immunity. Frontiers in Immunology 8: 1678. https://doi.org/10.3389/fimmu.2017.01678

Cong X, Xu W, Janton S, Henderson WA, Matson A, McGrath JM, Maas K, Graf J (2016) Gut microbiome developmental patterns in early life of preterm infants: Impacts of feeding and gender. PLoS One 11: 4. https://doi.org/10.1371/journal.pone.0152751

Couch CE, Epps CW (2022) Host, microbiome, and complex space: Applying population and landscape genetic approaches to gut microbiome research in wild populations. The Journal of Heredity 113(3): 221–234. https://doi.org/10.1093/jhered/esab078

Couch CE, Arnold HK, Crowhurst RS, Jolles AE, Sharpton TJ, Witczak MF, Epps CW, Beechler BR (2020) Bighorn sheep gut microbiomes associate with genetic and spatial structure across a metapopulation. Scientific Reports 10: 6582. https://doi.org/10.1038/s41598-020-63401-0

Coulter DP, Feiner ZS, Coulter AA, Diebel MW (2022) Using individual-based models to develop invasive species risk assessments by predicting species habitat suitability. Journal of Applied Ecology 59: 12. https://doi.org/10.1111/1365-2664.14304

Day T (2015) Information entropy as a measure of genetic diversity and evolvability in colonization. Molecular Ecology 24: 2073–2083. https://doi.org/10.1111/mec.13082

Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, et al. (2020) PICRUSt2 for prediction of metagenome functions. Nature Biotechnology 38(6): 685–688. https://doi.org/10.1038/s41587-020-0548-6

Dragičević P, Bielen A, Petrić I, Vuk M, Žučko J, Hudina S (2021) Microbiome of the successful freshwater invader, the signal crayfish, and its changes along the invasion range. Microbiology Spectrum 9: 2. https://doi.org/10.1128/Spectrum.00389-21

Eichmiller JJ, Hamilton MJ, Staley C, Sadowsky MJ, Sorensen PW (2016) Environment shapes the fecal microbiome of invasive carp species. Microbiome 4: 1. https://doi.org/10.1186/s40168-016-0190-1

Escalas A, Auguet J-C, Avouac A, Belmaker J, Dailianis T, Kiflawi M, Pickholtz R, Skouradakis G, Villéger S (2022) Shift and homogenization of gut microbiome during invasion in marine fishes. Animal Microbiome 4: 37. https://doi.org/10.1186/s42523-022-00181-0

Fackelmann G, Gillingham MA, Schmid J, Heni AC, Wilhelm K, Schwensow N, Sommer S (2021) Human encroachment into wildlife gut microbiomes. Communications Biology 4(1): 800. https://doi.org/10.1038/s42003-021-02315-7

Faith DP (1992) Conservation evaluation and phylogenetic diversity. Biological Conservation 61: 1–10. https://doi.org/10.1016/0006-3207(92)91201-3

Ferreiro A, Crook N, Gasparrini AJ, Dantas G (2018) Multiscale evolutionary dynamics of host-associated microbiomes. Cell 172: 6. https://doi.org/10.1016/j.cell.2018.02.015

Fischer ML, Hochkirch A, Heddergott M, Schulze C, Anheyer-Behmenburg HE, Lang J, Michler F-U, Hohmann U, Ansorge H, Hoffmann L, Klein R, Frantz AC (2015) Historical invasion records can be misleading: Genetic evidence for multiple introductions of invasive raccoons (*Procyon lotor*) in Germany. PLoS One 10: 5. https://doi.org/10.1371/journal.pone.0125441

Fischer ML, Salgado I, Beninde J, Klein R, Frantz AC, Heddergott M, Cullingham CI, Kyle CJ, Hochkirch A (2017) Multiple founder effects are followed by range expansion and admixture during the invasion process of the raccoon (*Procyon lotor*) in Europe. Diversity & Distributions 23: 4. https://doi.org/10.1111/ddi.12538

Fisher DN, Kilgour RJ, Siracusa ER, Foote JR, Hobson EA, Montiglio P-O, Saltz JB, Wey TW, Wice EW (2021) Anticipated effects of abiotic environmental change on intraspecific social interactions. Biological Reviews of the Cambridge Philosophical Society 96: 2661–2693. https://doi.org/10.1111/brv.12772

Fontaine SS, Mineo PM, Kohl KD (2022) Experimental manipulation of microbiota reduces host thermal tolerance and fitness under heat stress in a vertebrate ectotherm. Nature Ecology & Evolution 6: 4. https://doi.org/10.1038/s41559-022-01686-2

Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S (2017) The evolution of the host microbiome as an ecosystem on a leash. Nature 548: 43–51. https://doi.org/10.1038/nature23292

Frankham R (2005) Resolving the genetic paradox in invasive species. Heredity 94: 4. https://doi.org/10.1038/sj.hdy.6800634

Ghoul M, Mitri S (2016) The ecology and evolution of microbial competition. Trends in Microbiology 24: 833–845. https://doi.org/10.1016/j.tim.2016.06.011

Gillman SJ, McKenney EA, Lafferty DJ (2022) Human-provisioned foods reduce gut microbiome diversity in American black bears (Ursus americanus). Journal of Mammalogy 103(2): 339–346. https://doi.org/10.1093/jmammal/gyab154

Goddard-Dwyer M, López-Legentil S, Erwin PM (2021) Microbiome variability across the native and invasive ranges of the ascidian *Clavelina oblonga*. Applied and Environmental Microbiology 87: 2. https://doi.org/10.1128/AEM.02233-20

Gould AL, Zhang V, Lamberti L, Jones EW, Obadia B, Korasidis N, Gavryushkin A, Carlson JM, Beerenwinkel N, Ludington WB (2018) Microbiome interactions shape host fitness. Proceed-

103

ings of the National Academy of Sciences of the United States of America 115: 51. https://doi.org/10.1073/pnas.1809349115

Grosser S, Sauer J, Paijmans AJ, Caspers BA, Forcada J, Wolf JBW, Hoffman JI (2019) Fur seal microbiota are shaped by the social and physical environment, show mother–offspring similarities and are associated with host genetic quality. Molecular Ecology 28: 2406–2422. https://doi.org/10.1111/mec.15070

Halfvarson J, Brislawn CJ, Lamendella R, Vázquez-Baeza Y, Walters WA, Bramer LM, D'Amato M, Bonfiglio F, McDonald D, Gonzalez A, McClure EE, Dunklebarger MF, Knight R, Jansson JK (2017) Dynamics of the human gut microbiome in inflammatory bowel disease. Nature Microbiology 2: 5. https://doi.org/10.1038/nmicrobiol.2017.4

Henry LP, Bruijning M, Forsberg SKG, Ayroles JF (2021) The microbiome extends host evolutionary potential. Nature Communications 12: 5141. https://doi.org/10.1038/s41467-021-25315-x

Himler AG, Adachi-Hagimori T, Bergen JE, Kozuch A, Kelly SE, Tabashnik BE, Chiel E, Duckworth VE, Dennehy TJ, Zchori-Fein E, Hunter MS (2011) Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. Science 332: 254–256. https://doi.org/10.1126/science.1199410

Hutchins DA, Jansson JK, Remais JV, Rich VI, Singh BK, Trivedi P (2019) Climate change microbiology - problems and perspectives. Nature Reviews. Microbiology 17: 391–396. https://doi.org/10.1038/s41579-019-0178-5

Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bulletin de la Société Vaudoise des Sciences Naturelles 44: 223–270.

Jackson MA, Verdi S, Maxan M-E, Shin CM, Zierer J, Bowyer RCE, Martin T, Williams FMK, Menni C, Bell JT, Spector TD, Steves CJ (2018) Gut microbiota associations with common diseases and prescription medications in a population-based cohort. Nature Communications 9: 2655. https://doi.org/10.1038/s41467-018-05184-7

Jani AJ, Briggs CJ (2014) The pathogen *Batrachochytrium dendrobatidis* disturbs the frog skin microbiome during a natural epidemic and experimental infection. Proceedings of the National Academy of Sciences of the United States of America 111: E5049–E5058. https://doi.org/10.1073/pnas.1412752111

Jernelöv A (2017) Raccoons in Europe (Germany). In: Jernelöv A (Ed.) The long-term fate of invasive species: aliens forever or integrated immigrants with time? Springer International Publishing, 217–230. https://doi.org/10.1007/978-3-319-55396-2_15

Jones J, Armstrong D, Hoffmann R, Jones C (1983) Mammals of the Northern Great Plains. Mammalogy Papers, University of Nebraska State Museum. https://digitalcommons.unl.edu/museum-mammalogy/273

Kohl KD (2020) Ecological and evolutionary mechanisms underlying patterns of phylosymbiosis in host-associated microbial communities. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 375: 1798. https://doi.org/10.1098/rstb.2019.0251

Kolodny O, Schulenburg H (2020) Microbiome-mediated plasticity directs host evolution along several distinct time scales. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 375: 1808. https://doi.org/10.1098/rstb.2019.0589

Kołodziejczyk J, Fijarczyk A, Porth I, Robakowski P, Vella N, Vella A, Kloch A, Biedrzycka A (2025) Genomic investigations of successful invasions: The picture emerging from recent studies. Biological Reviews of the Cambridge Philosophical Society. https://doi.org/10.1111/brv.70005

Konopiński MK, Fijarczyk AM, Biedrzycka A (2023) Complex patterns shape immune genes diversity during invasion of common raccoon in Europe – selection in action despite genetic drift. Evolutionary Applications 16: 1. https://doi.org/10.1111/eva.13517

Koonin EV, Wolf YI (2012) Evolution of microbes and viruses: A paradigm shift in evolutionary biology? Frontiers in Cellular and Infection Microbiology 2: 119. https://doi.org/10.3389/fcimb.2012.00119

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology 79(17): 5112–5120. https://doi.org/10.1128/AEM.01043-13

Lavrinienko A, Tukalenko E, Kesäniemi J, Kivisaari K, Masiuk S, Boratyński Z, Mousseau TA, Milinevsky G, Mappes T, Watts PC (2020) Applying the Anna Karenina principle for wild animal gut microbiota: Temporal stability of the bank vole gut microbiota in a disturbed environment. The Journal of Animal Ecology 89: 11. https://doi.org/10.1111/1365-2656.13342

Lin H, Peddada SD (2020) Analysis of compositions of microbiomes with bias correction. Nature Communications 11: 1. https://doi.org/10.1038/s41467-020-17041-7

Liu H, Stiling P (2006) Testing the enemy release hypothesis: A review and meta-analysis. Biological Invasions 8: 7. https://doi.org/10.1007/s10530-005-5845-y

Louca S, Polz MF, Mazel F, Albright MBN, Huber JA, O'Connor MI, Ackermann M, Hahn AS, Srivastava DS, Crowe SA, Doebeli M, Parfrey LW (2018) Function and functional redundancy in microbial systems. Nature Ecology & Evolution 2: 936–943. https://doi.org/10.1038/s41559-018-0519-1

Lozupone C, Hamady M, Knight R (2006) UniFrac – An online tool for comparing microbial community diversity in a phylogenetic context. BMC Bioinformatics 7: 371. https://doi.org/10.1186/1471-2105-7-371

Macke E, Callens M, De Meester L, Decaestecker E (2017) Host-genotype dependent gut microbiota drives zooplankton tolerance to toxic cyanobacteria. Nature Communications 8: 1. https://doi.org/10.1038/s41467-017-01714-x

Marino CM, Pawlik JR, López-Legentil S, Erwin PM (2017) Latitudinal variation in the microbiome of the sponge *Ircinia campana* correlates with host haplotype but not anti-predatory chemical defense. Marine Ecology Progress Series 565: 53–66. https://doi.org/10.3354/meps12015

Martignoni MM, Kolodny O (2024) Microbiome transfer from native to invasive species may increase invasion risk. Proceedings. Biological Sciences 291: 2034. https://doi.org/10.1098/rspb.2024.1318

Maurice CF, Knowles SCL, Ladau J, Pollard KS, Fenton A, Pedersen AB, Turnbaugh PJ (2015) Marked seasonal variation in the wild mouse gut microbiota. The ISME Journal 9: 11. https://doi.org/10.1038/ismej.2015.53

McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer N, Mazmanian SK, Metcalf JL, Nealson K, Pierce NE, et al. (2013) Animals in a bacterial world, a new imperative for the life sciences. Proceedings of the National Academy of Sciences of the United States of America 110: 9. https://doi.org/10.1073/pnas.1218525110

Michler BA (2018) Koproskopische Untersuchungen zum Nahrungsspektrum des Waschbären *Procyon lotor* (L., 1758) im Müritz-Nationalpark (Mecklenburg-Vorpommern) unter Spezieller Berücksichtigung des Artenschutzes und des Endoparasitenbefalls. [Master's thesis]

Minard G, Tran F-H, Tran-Van V, Goubert C, Bellet C, Lambert G, Khanh HKL, Huynh T, Mavingui P, Valiente Moro C (2015) French invasive Asian tiger mosquito populations harbor reduced bacterial microbiota and genetic diversity compared to Vietnamese autochthonous relatives. Frontiers in Microbiology 6: 970. https://doi.org/10.3389/fmicb.2015.00970

Moeller AH, Shilts M, Li Y, Rudicell RS, Lonsdorf EV, Pusey AE, Wilson ML, Hahn BH, Ochman H (2013) SIV-induced instability of the chimpanzee gut microbiome. Cell Host & Microbe 14: 3. https://doi.org/10.1016/j.chom.2013.08.005

Nelson KE (2015) An update on the status of current research on the mammalian microbiome. ILAR Journal 56(2): 163–168. https://doi.org/10.1093/ilar/ilv033

Neu AT, Allen EE, Roy K (2021) Defining and quantifying the core microbiome: Challenges and prospects. Proceedings of the National Academy of Sciences of the United States of America 118: e2104429118. https://doi.org/10.1073/pnas.2104429118

Okarma H, Zalewski A, Bartoszewicz M, Biedrzycka A, Jedrzejewska E (2012) Szop pracz *Procyon lotor* w Polsce-ekologia inwazji. Studia i Materiały Centrum Edukacji Przyrodniczo-Leśnej 14: 4.

Perrier C, Rougemont Q, Charmantier A (2020) Demographic history and genomics of local adaptation in blue tit populations. Evolutionary Applications 13: 6. https://doi.org/10.1111/eva.13035

Phillips CD, Phelan G, Dowd SE, McDonough MM, Ferguson AW, Delton Hanson J, Siles L, Ordóñez-Garza N, San Francisco M, Baker RJ (2012) Microbiome analysis among bats describes influences of host phylogeny, life history, physiology and geography. Molecular Ecology 21: 2617–2627. https://doi.org/10.1111/j.1365-294X.2012.05568.x

Pickard JM, Zeng MY, Caruso R, Núñez G (2017) Gut microbiota: Role in pathogen colonization, immune responses and inflammatory disease. Immunological Reviews 279: 1. https://doi.org/10.1111/imr.12567

Ren T, Wu M (2016) PhyloCore: A phylogenetic approach to identifying core taxa in microbial communities. Gene 593: 330–333. https://doi.org/10.1016/j.gene.2016.08.032

Ritke ME (1990) Quantitative assessment of variation in litter size of the raccoon *Procyon lotor*. American Midland Naturalist 123: 2. https://doi.org/10.2307/2426567

Romeo C, Brenner E, Wauters LA, Alberdi A (2025) The role of microbiomes in animal invasions: A scoping review. NeoBiota 98: 335–360. https://doi.org/10.3897/neobiota.98.145939

Rosenberg E, Zilber-Rosenberg I (2018) The hologenome concept of evolution after 10 years. Microbiome 6: 1. https://doi.org/10.1186/s40168-018-0457-9

Rulison EL, Luiselli L, Burke RL (2012) Relative Impacts of Habitat and Geography on Raccoon Diets. American Midland Naturalist 168: 231–246. https://doi.org/10.1674/0003-0031-168.2.231

Sadoughi B, Schneider D, Daniel R, Schülke O, Ostner J (2022) Aging gut microbiota of wild macaques are equally diverse, less stable, but progressively personalized. Microbiome 10: 1. https://doi.org/10.1186/s40168-022-01283-2

Shade A, Handelsman J (2012) Beyond the Venn diagram: The hunt for a core microbiome. Environmental Microbiology 14: 4–12. https://doi.org/10.1111/j.1462-2920.2011.02585.x

Shannon CE (1948) A mathematical theory of communication. The Bell System Technical Journal 27: 379–423. https://doi.org/10.1002/j.1538-7305.1948.tb01338.x

Steelman SM, Chowdhary BP, Dowd S, Suchodolski J, Janečka JE (2012) Pyrosequencing of 16S rRNA genes in fecal samples reveals high diversity of hindgut microflora in horses and potential links to chronic laminitis. BMC Veterinary Research 8: 1–11. https://doi.org/10.1186/1746-6148-8-231

Stoffel MA, Acevedo-Whitehouse K, Morales-Durán N, Grosser S, Chakarov N, Krüger O, Nichols HJ, Elorriaga-Verplancken FR, Hoffman JI (2020) Early sexual dimorphism in the developing gut microbiome of northern elephant seals. Molecular Ecology 29: 2109–2122. https://doi.org/10.1111/mec.15385

Theis KR, Schmidt TM, Holekamp KE (2012) Evidence for a bacterial mechanism for group-specific social odors among hyenas. Scientific Reports 2: 615. https://doi.org/10.1038/srep00615

Tian L, Wang X-W, Wu A-K, Fan Y, Friedman J, Dahlin A, Waldor MK, Weinstock GM, Weiss ST, Liu Y-Y (2020) Deciphering functional redundancy in the human microbiome. Nature Communications 11: 1. https://doi.org/10.1038/s41467-020-19940-1

Troyer EM, Devitt SEC, Sunquist ME, Goswami VR, Oli MK (2014) Survival, recruitment, and population growth rate of an important mesopredator: The northern raccoon. PLoS One 9: 6. https://doi.org/10.1371/journal.pone.0098535

Uller T, Leimu R (2011) Founder events predict changes in genetic diversity during human-mediated range expansions. Global Change Biology 17: 3478–3485. https://doi.org/10.1111/j.1365-2486.2011.02509.x

Wemheuer F, Taylor JA, Daniel R, Johnston E, Meinicke P, Thomas T, Wemheuer B (2020) Tax4Fun2: Prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. Environmental Microbiome 15: 11. https://doi.org/10.1186/s40793-020-00358-7

Williams CL, Dill-McFarland KA, Vandewege MW, Sparks DL, Willard ST, Kouba AJ, et al. (2016) Dietary shifts may trigger dysbiosis and mucous stools in giant pandas (*Ailuropoda melanoleuca*). Frontiers in Microbiology 7: 661. https://doi.org/10.3389/fmicb.2016.00661

Williams CE, Hammer TJ, Williams CL (2024) Diversity alone does not reliably indicate the healthiness of an animal microbiome. The ISME Journal 18(1): wrae133. https://doi.org/10.1093/ismejo/wrae133

Willis AD (2019) Rarefaction, alpha diversity, and statistics. Frontiers in Microbiology 10: 2407. https://doi.org/10.3389/fmicb.2019.02407

Wong S, Rawls JF (2012) Intestinal microbiota composition in fishes is influenced by host ecology and environment. Molecular Ecology 21: 13. https://doi.org/10.1111/j.1365-294x.2012.05646.x

Wu J, Peters BA, Dominianni C, Zhang Y, Pei Z, Yang L, Ma Y, Purdue MP, Jacobs EJ, Gapstur SM, Li H, Alekseyenko AV, Hayes RB, Ahn J (2016) Cigarette smoking and the oral microbiome in a large study of American adults. The ISME Journal 10: 2435–2446. https://doi.org/10.1038/ismej.2016.37

Youngblut ND, Reischer GH, Walters W, Schuster N, Walzer C, Stalder G, Ley RE, Farnleitner AH (2019) Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. Nature Communications 10: 2200. https://doi.org/10.1038/s41467-019-10191-3

Zepeda-Paulo F, Ortiz-Martínez S, Silva AX, Lavandero B (2018) Low bacterial community diversity in two introduced aphid pests revealed with 16S rRNA amplicon sequencing. PeerJ 6: e4725. https://doi.org/10.7717/peerj.4725

Zhu L, Zhang Z, Chen H, Lamer JT, Wang J, Wei W, Fu L, Tang M, Wang C, Lu G (2020) Gut microbiomes of bigheaded carps and hybrids provide insights into invasion: A hologenome perspective. Evolutionary Applications 14: 3. https://doi.org/10.1111/eva.13152

Supplementary material 1

Results of alpha and beta diversity measures on samples from PL populations used to test whether the opportunistic mode of sample collection might influence population microbiomes

Link: https://doi.org/10.3897/neobiota.101.157515.suppl1

Supplementary material 2

Results that are not critical to understanding the general message of the article but provide deeper insight

Authors: Joanna Kołodziejczyk, Eric Hoffman, Aleksandra Biedrzycka

Data type: docx

Explanation note: All results are mentioned in the main text with direct citation.

Link: https://doi.org/10.3897/neobiota.101.157515.suppl2

Supplementary material 3

Results of the nearest-sequenced taxon analysis

Authors: Joanna Kołodziejczyk, Eric Hoffman, Aleksandra Biedrzycka

Data type: xlsx

Link: https://doi.org/10.3897/neobiota.101.157515.suppl3

Discussion

This doctoral thesis provides a comprehensive overview of mechanisms underlying successful invasion, combining three complementary approaches. First, I performed a systematic review of over one hundred original studies employing genomic methods to examine invasion and identified the key mechanisms facilitating invasive species success. As a next step, aiming to place the findings of the systematic review in the empirical context, I studied genomic footprints of selection in raccoons' populations from the native and invasive range. Finally, moving beyond the context of genomic diversity, I examined the composition and functional roles of the gut microbiome to assess its contribution to the raccoon's successful invasion in Central Europe.

The foundations of invasion genomics based on population genetic theory have been built and discussed in a number of reviews (Bock et al., 2015; Dlugosch & Parker, 2008; McGaughran et al., 2024; North et al., 2021). Nevertheless, the general picture, pointing and quantifying the key mechanisms, emerging from the results of recently published studies, has been lacking. As an introductory part of my dissertation (Article 1), I conducted a detailed, quantitative assessment of genomic studies that analyzed demographic and natural selection mechanisms during invasions to evaluate how they interplay in shaping genetic diversity that may facilitate the invasion process.

I identified a clear pattern indicating that genetic drift and genetic bottlenecks are frequently detected in invasive species. Nevertheless, reductions of genetic diversity are rarely maintained in invasive populations. When such reductions occur, in the majority of scenarios, they are counteracted by admixture of individuals originating from multiple introductions and interspecific gene flow. Introgression of advantageous genetic variants to invasive populations was, alongside admixture, identified as a factor facilitating adaptation, as both maintain the potential of rapid local adaptation. A subset of studies linked pre-introduction adaptation in the native range with invasion success, as favorable genetic variants from the native range often contributed to adaptation in the invasive range. The majority of articles reported the joint influence of selection and genetic drift or bottlenecks, while maintaining stable levels of genetic diversity. The results of the systematic review prove that adaptation usually occurs, even in the presence of demographic factors reducing genetic diversity and that admixture, introgression and genetic variation transferred from the native range may mitigate the effects of diversity loss and preserve the adaptive potential of invasive populations. The systematic classification of genomic regions under local selection revealed that they are related to specific biological functions, putatively facilitating invasion, such as temperature tolerance, immunity, metabolic pathways and insecticide resistance. Overall, those findings highlighted the most

commonly reported genetic mechanisms associated with biological invasions and provided the first comprehensive overview of the genetic processes facilitating invasion success.

The identification of mechanisms allowed for the detection of knowledge gaps and future directions of invasion genomics research, such as a pressing need for a broader application of time-point series genomic data to detect selection and demographic events at various stages of invasion to better understand how those processes shape population expansion. Incorporating the habitat and climate components into genomic analysis is essential to clarify the association of regions under selection with environmental features. The current studies often fail to use the full potential of whole-genome sequencing (WGS) data (Matheson & McGaughran, 2022), which represents genomic diversity not affected by the ascertainment bias linked to analyzing only a few representative marker loci, captures genome-wide variation, and therefore allows for reconstructing, e.g., invasion routes most accurately. Moreover, the use of WGS facilitates invasion studies by capturing rare alleles and long haplotypes, providing finer scale resolution needed to infer recent demography, identify selection footprints, a complete range of structural variants, and trace introgressed loci (North et al., 2021). Additionally, further development of comparative genomic studies may enable the identification of genome features that contribute to species' invasion abilities, as expansion of specific gene families or identification of structural variation contributing to invasion success.

To add an empirical context to findings from the systematic review, in the second part of my PhD thesis (Article 2), I aimed to identify adaptive changes facilitating the spread of one of the most rapidly expanding invasive mammals in Europe, the raccoon. I investigated genomic diversity, population structure, and patterns of adaptation in two geographically distant native populations and four invasive populations with distinct invasion histories. The findings supporting a lower level of genomic diversity and ongoing expansion followed by admixture within raccoons' invasive range are consistent with the conclusions drawn from the systematic review of invasion genomics research (Article 1). I showed that, as in the majority of invasions, the initial loss of genomic diversity is compensated by admixture among divergent populations.

Most importantly, the footprints of parallel selection between invasive populations and one of the native ones, established in similar environmental conditions, suggest habitat-specific local adaptations. On the contrary, the other native population inhabiting different climatic conditions experienced different selective pressures. Those results highlight the importance of habitat matching in the invasion process (Sherpa & Després, 2021). In line with the results of the systematic review, selection in invasive populations often operates on genes whose functions facilitate expansion and allow them to outcompete native species. In

raccoons, I detected selection footprints in genomic regions associated with reproduction, hormonal pathways, neurological processes, cognitive functions, locomotor system and environmental-related responses. These results show that the rapid selection on specific features may enhance raccoons' performance in the invasive range. Although most sites under selection, detected at the global level (among all studied populations) and those contrasting between invasive and native range, did not overlap, the gene ontology groups related to the same functions were found to be enriched in both kinds of comparisons. This indicates that selection may target functional outcomes rather than specific genetic variants (Wilson et al., 2025).

The results of the second part of my PhD dissertation are in line with the findings of the systematic review, demonstrating that the reduction of genomic diversity in raccoons' invasive populations does not prevent them from rapid adaptation. The parallel selection between populations from similar habitat types underlines the role of habitat matching between native and invasive ranges. At the molecular level, the success of raccoon as an invasive species appears to be driven by admixture restoring the genomic diversity, which further allowed local adaptation.

Finally, I expanded the hypotheses explaining species invasions to include factors beyond genetic diversity, through comparison of the native and invasive populations' microbiomes (Article 3). I focused on analyzing the differences in composition and functional profiles of raccoons' populations' gut microbiomes to assess their potential role in facilitating the species' successful spread in Central Europe. First, I hypothesized that demographic processes shaping the genetic differentiation of invasive populations would be reflected in the diversity of their gut microbiomes. I expected that the microbiomes of invasive populations were differentiated from the native ones, and indeed, I found a significant shift in the microbiomes of the two groups. This may either reflect diverse diet sources or be related to the loss and gain of microbial taxa during the invasion process, similar to how genetic diversity is shifted as a result of genetic drift during the establishment of invasive populations. Interestingly, invasive populations exhibited higher levels of inter- and intrapopulation microbiome diversity, likely driven by the acquisition of local microbial taxa during introduction. Contrary to expectations, I found no signs of decreased within-population microbiome diversity that could result from a population bottleneck. Conversely, the native range was characterized by a less diverse microbiome, similar between geographically distant populations, suggesting microbiome stability, but also possibly related to a more simplified, anthropogenic diet (Gillman et al., 2022; Moy et al., 2023). Although I found shifts in microbiome composition between ranges, gut microbiomes' functional profiles were

overlapping, suggesting that the same functions may be provided by different bacterial groups (Louca et al., 2018).

In summary, the obtained results show that, in the case of raccoons, the invasion process changes the microbiome composition, but it does not affect its function. The microbiome may contribute to various environmental adaptations, thus increasing the invasion success, but involving different host-microbiome associations.

Result summary and conclusions

Employing a three-step approach, I have performed a comprehensive investigation of genetic and non-genetic factors influencing biological invasions. As an outcome of my dissertation, I was able to i) quantify mechanisms shaping genetic diversity and occurrence of local adaptation in invasive populations, ii) uncover the processes shaping genetic diversity and enabling rapid local adaptation in invasive raccoons, and iii) reveal the potential role of the gut microbiome in facilitating raccoon invasion.

This integrative approach allowed for drawing the following key conclusions:

1) the so-called "genetic paradox" of invasive species is uncommon, as genetic drift and bottleneck rarely cause a significant genetic diversity decrease, and any reductions are typically mild and mitigated by gene flow between genetically divergent invasive populations (Articles 1 and 2),

2) even when a decrease in genomic diversity between native and invasive ranges is noted, it does not prevent adaptations in invasive populations (Articles 1 and 2),

3) most sites under selection are linked to traits promoting rapid expansion; however, they are not directly associated with the invasion, but rather reflect differences between populations from distinct climatic conditions, indicating the parallel selection in similar environmental conditions, and highlighting the role of habitat matching in species invasions (Article 2),

4) despite extensive genomic studies of invasion, further research requires a wider application of time-point series genomic data, the incorporation of habitat, climate and population fitness components into genomic analysis, as well as more frequent application of whole genome sequencing data (Articles 1 and 2),

5) the invasion process changes the raccoons' gut microbiota composition, but those shifts may also be a reflection of the between-populations differences in diet (Article 3),

6) invasive populations of raccoon possess highly diverse gut microbiota, with no obvious signs of instability and with functional capabilities comparable to the native populations, putatively facilitating the successful performance and spread of this invasive species (Article 3).

The results of my PhD thesis provide vital conclusions for improving invasive species management. First, I showed the frequency of admixture among distinct, genetically divergent populations during the invasions. Considering how often it is detected, it can be assumed that this process is central to restoring genetic diversity in invasive species. Therefore, even in the absence of genomic data, it is crucial to react to newly established invasive populations and prevent their spread and gene flow between independently established populations. At later

invasion stages, primary eradication efforts should be focused on regions where high migration rates have already been detected. Specifically, in the case of raccoons' invasion in Europe, it is already known that the complete eradication of the species is impossible, and management actions are undertaken mostly in vulnerable, biodiversity-rich areas (Cunze et al., 2025). While its broad climatic and habitat niche suggests it can invade a wide variety of habitats, the findings indicate the role of habitat matching between native and invasive ranges, even in the case of such cosmopolitan species as the raccoon. Habitat features should be taken into consideration while predicting areas at risk and identifying those requiring protection. Species preadapted to the particular conditions in their native range are more likely to succeed in similar conditions in the invasive range (Article 2). Climate change further complicates management by creating new introduction pathways, providing favorable climatic conditions, increasing the disturbance in the environment, leaving the empty niche, as native species may change their range, as a result of changing conditions. These make ecosystems more vulnerable to invasions (Colberg et al., 2024). Genomic data may improve invasive species management, not only by reconstructing the invasion history, but also by estimating the adaptive potential. As a result, it will be possible to plan effective management actions tailored to particular species.

References

Biedrzycka, A., Konopiński, M., Hoffman, E., Trujillo, A., & Zalewski, A. (2020). Comparing raccoon major histocompatibility complex diversity in native and introduced ranges: Evidence for the importance of functional immune diversity for adaptation and survival in novel environments. *Evolutionary Applications*, *13*(4), Article 4. https://doi.org/10.1111/eva.12898

Biedrzycka, A., Zalewski, A., Bartoszewicz, M., Okarma, H., & Jędrzejewska, E. (2014). The genetic structure of raccoon introduced in Central Europe reflects multiple invasion pathways. *Biological Invasions*, *16*(8), Article 8. https://doi.org/10.1007/s10530-013-0595-8

Bock, D. G., Caseys, C., Cousens, R. D., Hahn, M. A., Heredia, S. M., Hübner, S., Turner, K. G., Whitney, K. D., & Rieseberg, L. H. (2015). What we still don't know about invasion genetics. *Molecular Ecology*, *24*(9), Article 9. https://doi.org/10.1111/mec.13032

Bradley, B. A., Beaury, E. M., Gallardo, B., Ibáñez, I., Jarnevich, C., Morelli, T. L., Sofaer, H. R., Sorte, C. J. B., & Vilà, M. (2024). Observed and Potential Range Shifts of Native and Nonnative Species with Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, *55*(Volume 55, 2024), 23–40. https://doi.org/10.1146/annurev-ecolsys-102722-013135

Chiu, L., Bazin, T., Truchetet, M.-E., Schaeverbeke, T., Delhaes, L., & Pradeu, T. (2017). Protective microbiota: From localized to long-reaching co-immunity. *Frontiers in Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.01678

Chown, S. L., Hodgins, K. A., Griffin, P. C., Oakeshott, J. G., Byrne, M., & Hoffmann, A. A. (2015). Biological invasions, climate change and genomics. *Evolutionary Applications*, *8*(1), Article 1. https://doi.org/10.1111/eva.12234

Colberg, E. M., Bradley, B. A., Morelli, T. L., & Brown-Lima, C. J. (2024). Climate-smart invasive species management for 21st century global change challenges. *Global Change Biology*, *30*(10), e17531. https://doi.org/10.1111/gcb.17531

Couch, C. E., & Epps, C. W. (2022). Host, microbiome, and complex space: Applying population and landscape genetic approaches to gut microbiome research in wild populations. *Journal of Heredity*, *113*(3), 221–234. https://doi.org/10.1093/jhered/esab078

Coulter, D. P., Feiner, Z. S., Coulter, A. A., & Diebel, M. W. (2022). Using individual-based models to develop invasive species risk assessments by predicting species habitat suitability. *Journal of Applied Ecology*, *59*(12), Article 12. https://doi.org/10.1111/1365-2664.14304

Cunze, S., Schneider, G., Peter, N., & Klimpel, S. (2025). Linking patterns to processes: Using hunting bag data to classify raccoon (*Procyon lotor*) invasion stages in Germany since the 2000s. *Ecological Indicators*, *175*, 113568.

https://doi.org/10.1016/j.ecolind.2025.113568

Daly, E. Z., Chabrerie, O., Massol, F., Facon, B., Hess, M. C. M., Tasiemski, A., Grandjean, F., Chauvat, M., Viard, F., Forey, E., Folcher, L., Buisson, E., Boivin, T., Baltora-Rosset, S., Ulmer, R., Gibert, P., Thiébaut, G., Pantel, J. H., Heger, T., … Renault, D. (2023). A synthesis of biological invasion hypotheses associated with the introduction–naturalisation–invasion continuum. *Oikos*, *2023*(5), e09645. https://doi.org/10.1111/oik.09645

Dlugosch, K. M., Anderson, S. R., Braasch, J., Cang, F. A., & Gillette, H. D. (2015). The devil is in the details: Genetic variation in introduced populations and its contributions to invasion. *Molecular Ecology*, *24*(9), Article 9. https://doi.org/10.1111/mec.13183

Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, *17*(1), Article 1. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M., & Facon, B. (2016). Is there a genetic paradox of biological invasion? *Annual Review of Ecology, Evolution, and Systematics*, *47*(1), Article 1. https://doi.org/10.1146/annurev-ecolsys-121415-032116

Fischer, M. L., Hochkirch, A., Heddergott, M., Schulze, C., Anheyer-Behmenburg, H. E., Lang, J., Michler, F.-U., Hohmann, U., Ansorge, H., Hoffmann, L., Klein, R., & Frantz, A. C. (2015). Historical invasion records can be misleading: genetic evidence for multiple introductions of invasive raccoons (*Procyon lotor*) in Germany. *PLOS ONE*, *10*(5), Article 5. https://doi.org/10.1371/journal.pone.0125441

Gillman, S. J., McKenney, E. A., & Lafferty, D. J. R. (2022). Human-provisioned foods reduce gut microbiome diversity in American black bears (*Ursus americanus*). *Journal of Mammalogy*, *103*(2), 339–346. https://doi.org/10.1093/jmammal/gyab154

Haubrock, P. J., Turbelin, A. J., Cuthbert, R. N., Novoa, A., Taylor, N. G., Angulo, E., Ballesteros-Mejia, L., Bodey, T. W., Capinha, C., Diagne, C., Essl, F., Golivets, M., Kirichenko, N., Kourantidou, M., Leroy, B., Renault, D., Verbrugge, L., & Courchamp, F. (2021). Economic costs of invasive alien species across Europe. *NeoBiota*, *67*, 153–190. https://doi.org/10.3897/neobiota.67.58196

Henry, L. P., Bruijning, M., Forsberg, S. K. G., & Ayroles, J. F. (2021). The microbiome extends host evolutionary potential. *Nature Communications*, *12*(1), Article 1. https://doi.org/10.1038/s41467-021-25315-x

Jernelöv, A. (2017). Raccoons in Europe (Germany). In A. Jernelöv (Ed.), *The long-term fate of invasive species: Aliens forever or integrated immigrants with time?* (pp. 217–230). Springer International Publishing. https://doi.org/10.1007/978-3-319-55396-2_15

Kaňuch, P., Berggren, Å., & Cassel-Lundhagen, A. (2021). A clue to invasion success: Genetic diversity quickly rebounds after introduction bottlenecks. *Biological Invasions*, *23*(4), 1141–1156. https://doi.org/10.1007/s10530-020-02426-y

Kohl, K. D. (2020). Ecological and evolutionary mechanisms underlying patterns of phylosymbiosis in host-associated microbial communities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1798), 20190251. https://doi.org/10.1098/rstb.2019.0251

Kolodny, O., & Schulenburg, H. (2020). Microbiome-mediated plasticity directs host evolution along several distinct time scales. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1808), Article 1808. https://doi.org/10.1098/rstb.2019.0589

Lande, R., & Shannon, S. (1996). The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution*, *50*(1), Article 1. https://doi.org/10.1111/j.1558-5646.1996.tb04504.x

Lee, C. E. (2016). Evolutionary mechanisms of habitat invasions, using the copepod *Eurytemora affinis* as a model system. *Evolutionary Applications*, *9*(1), 248–270. https://doi.org/10.1111/eva.12334

Liu, H., & Stiling, P. (2006). Testing the enemy release hypothesis: A review and meta-analysis. *Biological Invasions*, *8*(7), Article 7. https://doi.org/10.1007/s10530-005-5845-y

Louca, S., Polz, M. F., Mazel, F., Albright, M. B. N., Huber, J. A., O'Connor, M. I., Ackermann, M., Hahn, A. S., Srivastava, D. S., Crowe, S. A., Doebeli, M., & Parfrey, L. W. (2018). Function and functional redundancy in microbial systems. *Nature Ecology & Evolution*, *2*(6), 936–943. https://doi.org/10.1038/s41559-018-0519-1

Matheson, P., & McGaughran, A. (2022). Genomic data is missing for many highly invasive species, restricting our preparedness for escalating incursion rates. *Scientific Reports*, *12*(1), 13987. https://doi.org/10.1038/s41598-022-17937-y

McGaughran, A., Dhami, M. K., Parvizi, E., Vaughan, A. L., Gleeson, D. M., Hodgins, K. A., Rollins, L. A., Tepolt, C. K., Turner, K. G., Atsawawaranunt, K., Battlay, P., Congrains, C., Crottini, A., Dennis, T. P. W., Lange, C., Liu, X. P., Matheson, P., North, H. L., Popovic, I., … Wilson, J. (2024). Genomic tools in biological invasions: Current state and future frontiers. *Genome Biology and Evolution*, *16*(1), Article 1. https://doi.org/10.1093/gbe/evad230

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, *7*(1), Article 1. https://doi.org/10.1111/eva.12137

Molnar, J. L., Gamboa, R. L., Revenga, C., & Spalding, M. D. (2008). Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, *6*(9), Article 9. https://doi.org/10.1890/070064

Moy, M., Diakiw, L., & Amato, K. R. (2023). Human-influenced diets affect the gut microbiome of wild baboons. *Scientific Reports*, *13*, 11886. https://doi.org/10.1038/s41598-023-38895-z

North, H. L., McGaughran, A., & Jiggins, C. D. (2021). Insights into invasive species from whole-genome resequencing. *Molecular Ecology*, *30*(23), Article 23. https://doi.org/10.1111/mec.15999

Parker, J. D., Torchin, M. E., Hufbauer, R. A., Lemoine, N. P., Alba, C., Blumenthal, D. M., Bossdorf, O., Byers, J. E., Dunn, A. M., Heckman, R. W., Hejda, M., Jarosík, V., Kanarek, A. R., Martin, L. B., Perkins, S. E., Pysek, P., Schierenbeck, K., Schlöder, C., van Klinken, R., … Wolfe, L. M. (2013). Do invasive species perform better in their new ranges? *Ecology*, *94*(5), Article 5. https://doi.org/10.1890/12-1810.1

Pickard, J. M., Zeng, M. Y., Caruso, R., & Núñez, G. (2017). Gut microbiota: Role in pathogen colonization, immune responses and inflammatory disease. *Immunological Reviews*, *279*(1), Article 1. https://doi.org/10.1111/imr.12567

Ritke, M. E. (1990). Quantitative assessment of variation in litter size of the raccoon *Procyon lotor*. *The American Midland Naturalist*, *123*(2), Article 2. https://doi.org/10.2307/2426567

Roe, A. D., Torson, A. S., Bilodeau, G., Bilodeau, P., Blackburn, G. S., Cui, M., Cusson, M., Doucet, D., Griess, V. C., Lafond, V., Paradis, G., Porth, I., Prunier, J., Srivastava, V., Tremblay, E., Uzunovic, A., Yemshanov, D., & Hamelin, R. C. (2019). Biosurveillance of forest insects: Part I—integration and application of genomic tools to the surveillance of non-native forest insects. *Journal of Pest Science*, *92*(1), 51–70. https://doi.org/10.1007/s10340-018-1027-4

Romeo, C., Brenner, E., Wauters, L. A., & Alberdi, A. (2025). The role of microbiomes in animal invasions: A scoping review. *NeoBiota*, *98*, 335–360. https://doi.org/10.3897/neobiota.98.145939

Roy, H. E., Pauchard, A., Stoett, P., Renard Truong, T., Bacher, S., Galil, B. S., Hulme, P. E., Ikeda, T., Sankaran, K., McGeoch, M. A., Meyerson, L. A., Nuñez, M. A., Ordonez, A., Rahlao, S. J., Schwindt, E., Seebens, H., Sheppard, A. W., & Vandvik, V. (2024). *IPBES Invasive Alien Species Assessment: Summary for Policymakers*. Zenodo. https://doi.org/10.5281/zenodo.11254974

Rulison, E. L., Luiselli, L., & Burke, R. L. (2012). Relative impacts of habitat and geography on raccoon diets. *The American Midland Naturalist*, *168*(2), 231–246. https://doi.org/10.1674/0003-0031-168.2.231

Salgado, I. (2018). Is the raccoon (*Procyon lotor*) out of control in Europe? *Biodiversity and Conservation*, *27*(9), Article 9. https://doi.org/10.1007/s10531-018-1535-9

Sherpa, S., & Després, L. (2021). The evolutionary dynamics of biological invasions: A multi-approach perspective. *Evolutionary Applications*, *14*(6), 1463–1484. https://doi.org/10.1111/eva.13215

Stange, M., Barrett, R. D. H., & Hendry, A. P. (2021). The importance of genomic variation for biodiversity, ecosystems and people. *Nature Reviews Genetics*, *22*(2), 89–105. https://doi.org/10.1038/s41576-020-00288-7

Wilson, J., Bieker, V. C., Boheemen, L. van, Connallon, T., Martin, M. D., Battlay, P., & Hodgins, K. A. (2025). Copy number variation contributes to parallel local adaptation in an invasive plant. *Proceedings of the National Academy of Sciences*, *122*(10), e2413587122. https://doi.org/10.1073/pnas.2413587122

Zepeda-Paulo, F., Ortiz-Martínez, S., Silva, A. X., & Lavandero, B. (2018). Low bacterial community diversity in two introduced aphid pests revealed with 16S rRNA amplicon sequencing. *PeerJ*, *6*, e4725. https://doi.org/10.7717/peerj.4725

Podziękowania

Dziękuję mojej promotorce, dr hab. Aleksandrze Biedrzyckiej, prof. IOP PAN, za zaufanie, możliwość ciągłego rozwoju, przekazanie mi cennej wiedzy i umiejętności. Dziękuję również za cierpliwość, konstruktywną krytykę i inspirowanie mnie do wejścia w świat nauki.

Dziękuję mojemu promotorowi pomocniczemu, dr hab. Maciejowi Konopińskiemu, prof. IOP PAN, za zaangażowanie, pomoc w analizach oraz wsparcie w rozwiązywaniu wielu zagwozdek bioinformatycznych.

Dziękuję koleżankom i kolegom z Instytutu Ochrony Przyrody, zwłaszcza tym z pokoju, za przyjazną atmosferę od pierwszych dni pracy.

Dziękuję także współautorom za wszystkie udzielone wskazówki i wkład w powstanie tej pracy.

Na koniec szczególne podziękowania kieruję do mojego narzeczonego, rodziców i przyjaciółek za cierpliwość, stałą obecność i ogromne wsparcie w trakcie przygotowywania tej pracy.

Acknowledgements

I would like to thank my supervisor, dr hab. Aleksandra Biedrzycka, prof. IOP PAN for her trust, for allowing me to continuously develop, and for sharing the valuable knowledge and skills with me. I would also like to thank her for her patience, constructive criticism, and for inspiring me to enter the world of science.

I would like to thank my auxiliary supervisor, dr hab. Maciej Konopiński, prof. IOP PAN, for his commitment, help with analyses, and support in solving many bioinformatic puzzles.

I would like to thank my colleagues from the Institute of Nature Conservation, especially those in my room, for the friendly atmosphere from my very first days at work.

I would also like to thank my co-authors for all their guidance and contribution to this work.

Finally, I would like to express my special thanks to my fiancé, parents, and friends for their patience, constant presence, and huge support during the preparation of this work.

Kraków, 17.11.2025

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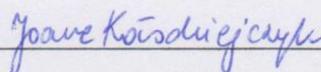
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OŚWIADCZENIE

Oświadczam, że:

- w pracy *Kołodziejczyk, J., Fijarczyk, A., Porth, I., Robakowski, P., Vella, N., Vella, A., Kloch, A., & Biedrzycka, A. (2025). Genomic investigations of successful invasions: The picture emerging from recent studies. Biological Reviews, 100(3), 1396–1418.* mój udział polegał na: analizie 1/8 artykułów włączonych do przeglądu, współudziale w zbieraniu i analizie danych, opracowaniu graficznym wyników oraz współudziale w pisaniu oraz korekcie manuskryptu,

- w pracy *Kołodziejczyk, J., Konopiński, M.K., Hoffman, E.A., Biedrzycka, A. (202x). Signatures of differential local adaptation indicate environmental matching with specific native-range habitats in invasive raccoons (Procyon lotor)* mój udział polegał na: współudziale w zaplanowaniu koncepcji i metodyki badań, ekstrakcji DNA z próbek z zasięgu inwazyjnego, wykonaniu bibliotek do sekwencjonowania RAD, współudziale w analizie wyników oraz współudziale w pisaniu oraz korekcie manuskryptu,

- w pracy *Kołodziejczyk, J., Hoffman, E., Biedrzycka, A. (2025). Native and invasive raccoon populations host distinct microbiomes that may facilitate invasion. NeoBiota 101: 45-71.* mój udział polegał na: współudziale w zaplanowaniu koncepcji i metodyki badań, ekstrakcji DNA z próbek z zasięgu inwazyjnego, wykonaniu bibliotek do sekwencjonowania, przeprowadzeniu analiz bioinformatycznych, przedstawieniu wyników oraz współudziale w pisaniu oraz korekcie manuskryptu.
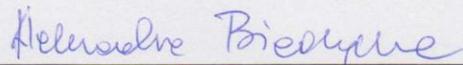
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- w pracy *Kołodziejczyk, J., Hoffman, E., Biedrzycka, A. (2025). Native and invasive raccoon populations host distinct microbiomes that may facilitate invasion. NeoBiota 101: 45-71.* mój udział polegał na: pozyskaniu finansowania, współudziale w zaplanowaniu koncepcji badań, współudziale w pisaniu manuskryptu i jego korekcie.

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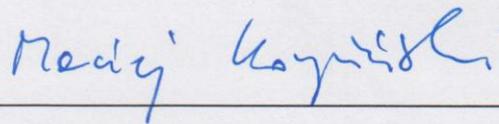(czytelny podpis współautora)

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CO-AUTORSHIP DECLARATION

I declare that:

- in the manuscript *Kołodziejczyk, J., Konopiński, M.K., Hoffman, E.A., Biedrzycka, A. (202x). Signatures of differential local adaptation indicate environmental matching with specific native-range habitats in invasive raccoons (Procyon lotor)* my contribution consisted of management of sample collection and DNA extraction from native raccoon populations, participation in manuscript correction,

- in the manuscript *Kołodziejczyk, J., Hoffman, E., Biedrzycka, A. (2025). Native and invasive raccoon populations host distinct microbiomes that may facilitate invasion. NeoBiota 101: 45-71*. I contributed by the management of sample collection and DNA extraction from native raccoon populations, and participation in manuscript correction.

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