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ORIGINAL RESEARCH

Without the locals' aid: no evidence for a role of admixture in the colonisation success of Italian wall lizards

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Abstract
The successful establishment of small founding populations introduced into novel environments often represents a paradox, given the genetic challenges they face. Genetic admixture, whether intra- or interspecific, may offer a solution by enhancing the genetic diversity and adaptability of the population. Here, we explore the role of genetic admixture in the rapid establishment and adaptation of the Italian wall lizard (*Podarcis siculus*) introduced on Pod Mrčaru, a small islet in the Adriatic Sea. Introduced in 1971 in a small group of ten individuals from a nearby island, this population rapidly adapted to its new environment, outcompeting the native Dalmatian wall lizard (*Podarcis melisellensis*) and exhibiting striking ecological and phenotypic shifts. Using whole-genome sequencing data from *P. siculus* populations on Pod Mrčaru and neighbouring islands and from *P. melisellensis*, we investigated population structure, admixture, and gene flow to test whether inter- or intraspecific genetic exchange contributed to the successful establishment and divergence of the introduced population. Despite the sympatric presence of *P. melisellensis* during the introduction of *P. siculus* on Pod Mrčaru, and thus the opportunity for genetic exchange, we found no evidence of hybridisation between the two species. Even amongst neighboring island populations of *P. siculus*, we observed only limited gene flow, suggesting relatively independent evolution of the populations since their establishment on the islands. These findings highlight the potentially significant roles of distinct selective pressures and/or ecological and phenotypic plasticity, rather than genetic exchange, in driving the population's rapid adaptation to a novel environment.

Keywords *Podarcis* · Island populations · Genomics · Hybridization · WGS

Introduction

It happens that small propagules of animals or plants reach areas well outside their native range, and, against all odds, establish prosperous populations. In doing so, they may outcompete resident species, with a much longer history of local adaptation. This phenomenon, well-known to invasion biologists (e.g. Estoup et al. 2016) and those studying island communities (e.g. Sax and Brown 2000), presents a paradox, because colonising populations tend to be small, have limited genetic diversity, and have no experience with local environmental challenges — features that should jeopardise their establishment success.

Genetic rescue has been suggested as a possible way out of the conundrum (Whiteley et al. 2015). By allowing new allelic combinations, the assimilation of genetic material from other lineages into the colonisers' genome may lead to heterosis (Facon et al. 2005) and increased adaptability (Verhoeven et al. 2010), promoting population viability in the shorter and longer term (Rius and Darling 2014). In situations where a new area is invaded repeatedly by different lineages of the same species, intraspecific admixture is common (e.g. Kolbe et al. 2004; Vilatersana et al. 2016; van Boheemen et al. 2017), although not inevitable (e.g.

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Ordóñez et al. 2013). Several studies have reported on the benefits of such intraspecific admixture for individual fitness (e.g. Keller and Taylor 2010) and adaptability (e.g. Qiao et al. 2019). But again, other studies have found no or even negative effects (e.g. Chapple et al. 2013; Barker et al. 2019).

New alleles may also come into the colonising population across a species boundary, by hybridisation and introgression (e.g. Ryan et al. 2009; Hovick and Whitney 2014; Rosinger et al. 2021). Although interspecific admixture is often associated with negative consequences, such as the transfer of deleterious variation (Harris and Nielsen 2016), outbreeding depression (Beauclerc et al. 2013), anatomical anomalies (Orr 1990), high mortality rates (Zorenko et al. 2016), reduced reproductive output (Pampoulie et al. 2021), and even extinction (Todesco et al. 2016), it can also have beneficial effects. Hybrid individuals not seldomly show increased fitness ('hybrid vigour', see meta-analysis in Hovick and Whitney 2014), which can aid the survival and establishment of a founder population. Like intraspecific admixture, hybridisation could boost genetic variability (e.g. Gaskin 2017), reduce inbreeding depression (e.g. Van Vianen et al. 2015), improve reproductive output (e.g. Williams et al. 2019) and bring adaptive alleles to the colonising gene pool (e.g. Touchard et al. 2024).

Here, we investigate whether genetic admixture has played a role in the establishment of a population of the Italian wall lizard (*Podarcis siculus,* Rafinesque-Schmaltz, 1810) on a small islet in the Adriatic Sea (Pod Mrčaru, Croatia). On 14 August 1971, Eviatar Nevo and co-workers took five male and five female specimens of *P. siculus* from the islet of Pod Kopište and released them onto Pod Mrčaru, 4.5 km to the northeast (Nevo et al. 1972). At that time, Pod Mrčaru was inhabited by the Dalmatian wall lizard *Podarcis melisellensis* and the sharp-snouted rock lizard *Dalmatolacerta oxycephala*. When we visited the islet in 2007, we found only *P. siculus* (and the occasional *D. oxycephala*), suggesting that in ca. 30 generations, the descendants of the 10 introduced Italian wall lizards had taken over the island and outcompeted the local Dalmatian wall lizards (Herrel et al. 2008). The study system of Pod Kopište and Pod Mrčaru has become a well-known example in the field of evolutionary biology, not the least because of some striking differences that arose between the source population (on Pod Kopište) and the newly founded one (on Pod Mrčaru) on a very short timescale. Individuals of the two populations differ in average body size and limb proportions, sprint speed, running endurance, and antipredator behaviour (Vervust et al. 2007). Individuals from the newly established population on Pod Mrčaru behave more aggressively, and bite harder (Vervust et al. 2009a). They also exhibit higher levels of fluctuating asymmetry (Vervust et al. 2009b). Most notable, however, is the difference in diet: whilst the lizards from the source population maintain a typical *Podarcis* diet, dominated by arthropods, the Pod Mrčaru lizards have shifted to a much more herbivorous diet (Herrel et al. 2008). This dietary change seems to have come with a suite of morphological and physiological changes to the head (Herrel et al. 2008; Taverne et al. 2023), and the digestive tract and gut microbiome (Herrel et al. 2008; Vervust et al. 2010; Wehrle et al. 2020; Lemieux-Labonté et al. 2022). A recent genomic study revealed that at least some of the phenotypic differences between the two populations are reflected in the variation at the level of the genome (Sabolić et al. 2024). In addition, outlier loci found amongst the populations were linked with possible diet-based adaptation candidate genes (Sherpa et al. 2023). However, in both studies, the applied reduced representation genomic methods did not permit the informative conclusion of whether intra- or interspecific gene flow facilitated this rapid adaptation.

In this study, we test whether interspecific admixture has contributed to the (remarkably fast) changes observed in the Pod Mrčaru population. *Podarcis melisellensis*, inhabiting Pod Mrčaru at the time of the introduction, is estimated to have diverged from *P. siculus* around 12 to 18 Mya (Poulakakis et al. 2005; Tonini et al. 2016; Yang et al. 2021). Although reproductive isolation is usually thought to occur faster than that (Sasa et al. 1998), notable exceptions are known from all vertebrate classes (e.g. mammals: Skidmore et al. 1999, turtles: Brito et al. 2020, fish: Káldy et al. 2020, birds: Alfieri et al. 2023), including lizards (Jančúchová-Lásková et al. 2015). In addition, the genus *Podarcis* has a history of rampant introgression and *P. siculus* has been described as a genetic mosaic, with up to 24% of its genome originating from congeneric species (Yang et al. 2021). These include lineages from the Iberian subclade (diverged from *P. siculus* ~ 4.5 Mya), but also from the Sicilian-Maltese and Balkan subclades (> 17Mya). Recent natural hybridisation within the *Podarcis* genus has been reported from the Aeolian and Aegedian Islands (*P. wagleriana* x *P. siculus*, Capula 1993; *P. raffonei* x *P. siculus*, Ficetola et al. 2024, Paris et al. 2024), southern Sardinia (*P. tiliguerta* x *P. siculus*, Capula 2002) and the Iberian peninsula (amongst species of the *P. hispanicus* complex, Pinho et al. 2009, Caeiro-Dias et al. 2021, Gaczorek et al. 2023). Interestingly, in an early allozyme study of Pod Mrčaru *P. melisellensis*, Gorman et al. (1975) detected three alleles that were not found in any of 11 other *P. melisellensis* sampled on neighbouring islands. Two of these were electrophoretically identical with alleles found in *P. siculus*, and the third one was also found in *D. oxycephala*. Although they acknowledge that the evidence is circumstantial, Gorman et al. (1975) hint at the possibility that the species may have interbred, despite the genetic distance.

To investigate the possibility of admixture between *P. siculus* and *P. melisellensis* on Pod Mrčaru, we examine the genomic structure of this and other island populations in the Adriatic Sea. By assessing population structure, admixture signals, and gene flow, we aim to determine whether there is any evidence of genetic exchange between the two species, and explore its potential role, if present, in shaping the evolution of the Pod Mrčaru *P. siculus* population.

Methods

Lizard tail tips (~ 0.5 cm) were collected from individuals on Sušac ($N = 12$), Kopište ($N = 13$), Pod Kopište ($N = 14$), and Pod Mrčaru ($N = 14$) in April 2023, with additional samples from Bijelac ($N = 5$) collected in 2016 (Fig. 1a). After DNA extraction, library preparation and paired-end 150-bp sequencing on a DNBSEQ platform were carried

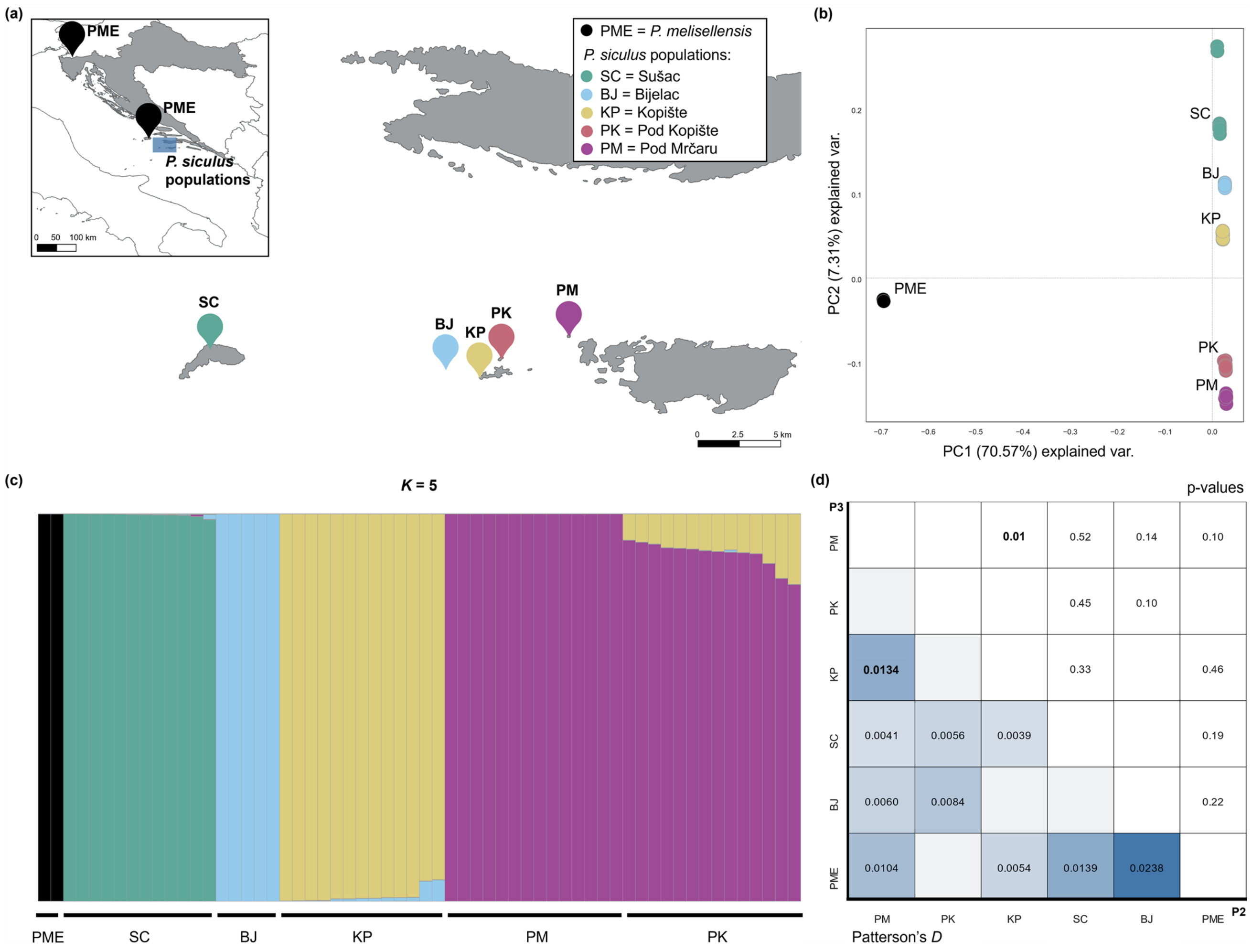

Fig. 1 **a** Detailed map of the sampled *P. siculus* populations of the Lastovo archipelago (coordinates: 42°45′23.0″N 16°52′57.0″E), with a broader geographical map of Croatia (grey), Italy, and surrounding countries in the top left to provide context, including the location where the *P. melisellensis* (PME) individuals were sampled (NCBI accessions: ERR13317837 (Feiner et al. 2025a), sampling location: Vis, Croatia; SRR14009399 (Yang et al. 2021), Hrastovlje, Slovenia) and a blue frame indicating the location of the zoomed-in study area. The legend shows the colour scheme and abbreviations used. SC = Sušac ($N = 12$), KP = Kopište ($N = 13$), BJ = Bijelac ($N = 5$), PK = Pod Kopište ($N = 14$), PM = Pod Mrčaru ($N = 14$). **b** Population genetic structure as indicated by the PCA plot. Each dot represents an individual. PC1 explains 70.57% of the observed variance and PC2 7.31%. **c** ADMIXTURE plot with 5 genetic clusters (K), the grouping with the lowest CV error. Each coloured bar represents a *P. siculus* individual from the respective populations. The *P. melisellensis* individuals are shown in black. **d** Heatmap of the Dsuite results (Dtrios command with *A. bedriagae* as the outgroup). The lower left triangle shows Patterson's D values or the ABBA-BABA statistic, indicating evidence for gene flow between population or species pairs (x-axis: P2, y-axis: P3, according to Malinsky et al. 2021). Higher D-values are coloured darker blue. The upper left triangle shows the respective p-values, indicating whether the D-values are significantly different from zero. Significant pairs of Patterson's D and p-values are indicated in bold: a signal of excess allele sharing between Pod Mrčaru (P2) and Kopište (P3) relative to the Bijelac population (P1) (Patterson's $D = 0.0134$, $p = 0.013$, z-score = 2.495, f4-ratio = 0.218)

out by BGI (Shenzhen, China). The quality of the sequence reads was inspected using FASTQC v0.11.9. In addition, FASTQ files available on NCBI of *P. melisellensis* (two samples, run accessions: SRR14009399, Yang et al. 2021, and ERR13317837, Feiner et al. 2025a) and *Archaeolacerta bedriagae* (one sample, run accession: SRR14009418) (outgroup, Yang et al. 2021) were downloaded and included in the dataset (Table S1). The filtered reads were mapped to the *P. siculus* reference genome rPodSic1.hap1.1 (assembly accession: GCA_964188175.1, Feiner et al. 2025b) using bwa-mem v0.7.17 (Li and Durbin 2009). The resulting sequence alignment files (BAM format) were then sorted, indexed, and checked using SAMtools v1.14 (Li et al. 2009) and deepTools2.0 (Ramírez et al. 2016). Variant calling was performed using BCFtools v1.14 (Danecek et al. 2021). Variants were filtered to exclude sites with over 10% mapped reads at mapping quality zero and with an overall mapping quality below 50. In addition, sites with abnormally deviant sequencing depths (> 2.8 standard deviation across all samples) and sites with biassed read depth of the reference and alternative alleles (PHRED score > 20) were also removed. Lastly, only sites with less than 20% missing genotypes and biallelic single-nucleotide polymorphisms (SNPs) were retained.

To assess whether there was admixture between the original *P. melisellenis* population and the introduced *P. siculus* population on the island of Pod Mrčaru, and possibly also amongst the other insular populations in the Adriatic Sea, we explored population structure, admixture and gene flow signals using the generated SNP dataset. If hybridisation occurred between the two species during establishment, we would expect to detect signatures of the species *P. melisellensis* within the genomes of *P. siculus* individuals from Pod Mrčaru in our analyses below. The *P. melisellensis* samples used in this study do not originate from the original population on Pod Mrčaru or its satellite islets (clade divergence estimates 1.2—1.9 Mya, Podnar et al. 2004; Fig. 1). However, the hypothesised hybridisation event is thought to have occurred only ~ 40 generations ago. At this timescale, introgressed genomic regions, even after shortening due to recombination (Aguillon et al. 2022), are expected to remain relatively large, especially if they are adaptive (Shchur et al. 2020; Veller et al. 2023). Thus, we expect the signal of introgression to be highly detectable with genome-wide SNP data (Caeiro-Dias et al. 2021; DeVos et al. 2023), even using genomically diverged *P. melisellensis* clades.

First, we explored population genetic structure using PLINK 2.0 (Chang et al. 2015). For this, we filtered the SNP dataset to prune SNPs in linkage disequilibrium (LD), using a sliding-window approach, excluding SNP pairs with $r^2 > 0.2$ and minor allele frequency of 0.01, within 50-SNP windows with a 10-SNP step size, and performed a principal component analysis (PCA). In this PCA, evidence of hybridisation between *P. melisellensis* and *P. siculus* would be observed as intermediate positions of Pod Mrčaru *P. siculus* samples along principal components dominated by allele frequency differences between the two species. Evidence of gene flow amongst *P. siculus* insular populations would be indicated by the clustering of individuals from geographically or ancestrally distinct populations in proximity to each other within the PCA space, deviating from patterns explained solely by geographical proximity or prior population assignments.

Second, we performed a population admixture analysis using ADMIXTURE v1.3.0 (Alexander et al. 2009) to identify ancestral genetic clusters ($K = 1$–7) in our dataset. For this, we used the same pruned SNP dataset as above and converted it to binary format (.bed) as input (Chang et al. 2015). The best number of genetic groups (K) was selected based on the lowest corresponding cross-validation (CV) error. In the ADMIXTURE analysis, evidence of hybridisation would be observed if Pod Mrčaru *P. siculus* individuals exhibit admixture proportions indicative of genetic contributions from *P. melisellensis*, represented as a distinct ancestral genetic component. This pattern would contrast with other *P. siculus* insular populations, which would lack or display reduced proportions of this component.

Lastly, to investigate potential gene flow amongst the insular populations and between *P. siculus* and *P. melisellensis*, we calculated Patterson's D (also known as the ABBA-BABA statistic) using Dsuite (command Dtrios) (Malinsky et al. 2021). *Archaeolacerta bedriagae* was used as the outgroup in this analysis. The Patterson's D statistic examines asymmetries in allele-sharing patterns across closely related populations or species. A positive D statistic indicates an excess of shared derived alleles between species/populations pair P2 and P3 compared to P1 and P3, suggesting gene flow between P2 and P3 (see also Fig. 1 in Malinsky et al. 2021). Evidence of admixture between *P. melisellensis* and Pod Mrčaru *P. siculus* would manifest as significant positive D values, reflecting asymmetric sharing of derived alleles between these populations compared to other *P. melisellensis* and insular *P. siculus* pairs. Similarly, positive D values amongst other *P. siculus* island populations would indicate gene flow, as evidenced by excess allele sharing amongst those population pairs. In case significant D values were found, we followed up on these results by running Dinvestigate (SNP window size: 250, SNP step size: 100), which calculates sliding-window statistics of excess allele-sharing (fdM) for specific trios (Malinsky et al. 2021). Here, large genomic regions with asymmetric, elevated fdM values would indicate recent introgression (Malinsky et al. 2015, 2021). All results were plotted using matplotlib (Hunter 2007).

Results

We whole-genome sequenced 58 Italian wall lizards to a median of 15.6-fold genomic coverage. The average percentage of reads mapped to the reference genome was 99.46% (± 0.18) for the *P. siculus* samples, and 98.05% (± 0.78) and 95.80%, respectively, for *P. melisellensis* and *A. bedriagae* (Table S1). Following variant calling and filtering, ~ 86 million SNPs were retained and used in subsequent analyses. PCA of the genomes showed a high divergence between *P. melisellensis* and all five *P. siculus* populations (PC1 explaining 70.57% of the observed variation) (Fig. 1b). PC2 reflected the divergence amongst the *P. siculus* insular populations (explaining 7.31% of the observed variance), with each island forming a distinct cluster. As expected, the individuals from Pod Kopište and Pod Mrčaru clustered closest together (Fig. 1b). ADMIXTURE analysis of the samples and CV error suggested five distinct genetic clusters (Fig. 1c and Table S2). The results revealed no sign of admixture between *P. melisellensis* and the *P. siculus* island populations, including Pod Mrčaru. However, varying and overall small degrees of shared ancestry are present amongst *P. siculus* populations, mostly amongst Kopište, Pod Kopište, and Pod Mrčaru (Fig. 1c).

The calculated Patterson's *D* between pairs of *P. melisellensis* and *P. siculus* populations indicated no significant patterns of excess allele sharing between the two species (all $p > 0.05$) (Fig. 1d). Also, there were very few signs of gene flow amongst the *P. siculus* populations, as indicated by the small, non-significant *D*-statistics (Table S3). Only a signal of excess allele sharing between Kopište and Pod Mrčaru, relative to the Bijelac population, was detected (P1: BJ, P2: PM, P3: KP; Patterson's $D = 0.0134$, $p = 0.013$, z-score = 2.495, f4-ratio = 0.218) (Fig. 1d and Table S3). Genome-wide *f*dM for this trio was equal to 0.00869, with no clear genomic regions of elevated *f*dM (Fig. 1S).

Discussion

Here, we investigated population genetic structure, admixture signals, and gene flow among *P. melisellensis* and different island populations of *P. siculus* in the Adriatic sea, to assess whether *P. siculus* on Pod Mrčaru might have interbred with *P. melisellensis* during the early stages of their establishment on the island. Despite the potential for genetic exchange given the sympatric presence of *P. melisellensis* when *P. siculus* was introduced (Nevo et al. 1972), our analyses showed no signs of introgression between the two species. Specifically, PCA and ADMIXTURE consistently assigned the Pod Mrčaru individuals to the *P. siculus* cluster, separating them from the *P. melisellensis* individuals and indicating no admixed genotypes amongst species. However, both PCA (Novembre and Stephens 2008; McVean 2009; Privé et al. 2020; Elhaik 2022) and ADMIXTURE (Lawson et al. 2018; Wang 2022) can be sensitive to unbalanced sample sizes, as is the case in this dataset (2 *P. melisellensis* individuals vs. 58 *P. siculus* individuals). Nevertheless, the first principal component and ADMIXTURE strongly separate the two species, suggesting that interspecific divergence is the dominant signal in the dataset, not artefacts of sample imbalance. In addition, we also calculated *D* statistics, which are robust to sample size differences because they are based on allele frequency correlations rather than individual-based clustering (Durand et al. 2011; Malinsky et al. 2021). These statistics also allow detecting admixture when the original admixing population is not sampled (as is the case in this dataset) (Durand et al. 2011). The calculated Patterson's *D* likewise revealed no significant excess of allele sharing between *P. siculus* from Pod Mrčaru and *P. melisellensis*.

The lack of evidence for hybridisation between *P. siculus* and *P. melisellensis* on Pod Mrčaru in our analyses suggests that the observed ecological and phenotypic shifts in the *P. siculus* population (Herrel et al. 2008; Wehrle et al. 2020; Lemieux-Labonté et al. 2022; Taverne et al. 2023; Sabolić et al. 2024) likely arose independently of interspecific gene flow.

While interspecific gene flow appears absent, intraspecific gene flow into the population of *P. siculus* on Pod Mrčaru could have facilitated the rapid adaptation (e.g. Qiao et al. 2019). However, our analyses also showed a clear population structure amongst *P. siculus* populations, including those from geographically close islands, with limited evidence of recent admixture. Each insular *P. siculus* population exhibited predominantly unique genetic components, with only small shared ancestry signals. Most notable, however, is the seemingly admixed nature of the population from Pod Kopište, which, more specifically, represents mostly a mixture of Pod Mrčaru and Kopište, despite it being the source of the lizard population on Pod Mrčaru. This is likely the result of the underlying algorithm of ADMIXTURE (see Fig. 2b in (Lawson et al. 2018) for a similar example of recent gene flow and the resulting barplot). The algorithm is biassed towards calling allelic state combinations that are shared by many individuals as ancestral (Alexander et al. 2009; Lawson et al. 2018). Because Pod Mrčaru underwent a relatively recent bottleneck deriving from the Pod Kopište population, the algorithm assigns the same genetic cluster to both, but because of the strong signature of this bottleneck (i.e. more homogeneous allelic states), Pod Mrčaru is assigned a higher proportion of this genetic cluster, thereby making it more distinct than Pod Kopište. The latter is then described as allelic states similar to Pod Mrčaru but retaining some allelic diversity from the most closely related genetic cluster, in this case, the Kopište population. Thus, this reflects phylogenetic distance rather than admixture amongst the populations (Lawson et al. 2018). The samples from Bijelac are most likely showing a similar phenomenon, as this is a small, barren island (0.005 km^2) harbouring a very small and inbred population of lizards (Sabolić et al. 2024, preliminary results), which the algorithm then assigns as a distinct cluster which shows small signs of admixture with the other populations, because of the strong genomic signature of the bottleneck (Fig. 1c).

This pattern of limited gene flow among *P. siculus* populations is further corroborated by the small Patterson's *D*-values and the accompanying *p*-values and f4-ratios. A slight excess of allele sharing was found between the populations on Kopište and Pod Mrčaru populations, relative to the Bijelac population. However, there were no clear, asymmetrically elevated *f*dM values along the genome, suggesting no recent introgression. This pattern in *D* statistics likely reflects the historical establishment of these populations rather than recent gene flow. Repeated subsampling from the original founding population at different times, combined with genetic drift in these small founder populations, has likely led to Kopište and Pod Mrčaru being more genetically similar to each other than Kopište and Bijelac. If recent gene flow (before the introduction event) were responsible for this similarity, we would also expect to see excess allele sharing between Kopište and Pod Kopište, given that the individuals of Pod Mrčaru are descendants of the Pod Kopište population. Or, if gene flow from Kopište into the Pod Mrčaru population occurred within the last 40 generations, we would expect to see large blocks of elevated *f*dM values along the genome. However, since these patterns are not found, the observed genetic similarity is more likely due to historical factors rather than ongoing or recent migration between Kopište and Pod Mrčaru.

This lack of large shared ancestry signals thus suggests that these insular *P. siculus* populations have evolved relatively independently since their establishment on each island, as other studies have also suggested (Sherpa et al. 2023; Sabolić et al. 2024). For example, Sabolić et al. (2024) reported undetectable levels of recent migration events amongst these populations.

Overall, our findings suggest that the rapid changes in morphology, diet, and behaviour in the Pod Mrčaru *P. siculus* population likely resulted from selection on standing genetic variation and/or phenotypic plasticity (Sherpa et al. 2023; Sabolić et al. 2024), rather than from hybrid vigour or the incorporation of adaptive alleles from *P. melisellensis* or surrounding island populations. This is intriguing in the broader context of island invasions and the role of genetic admixture in facilitating adaptation, as genetic exchange is often expected to generate new allelic combinations that enhance adaptability and promote population persistence (e.g. Rius and Darling 2014; Whiteley et al. 2015). Our results, however, indicate that *P. siculus* successfully established an ecologically diverged population on Pod Mrčaru without interspecific or intraspecific admixture and in the face of reduced genetic diversity because of the small founding population (Sherpa et al. 2023; Sabolić et al. 2024). Even though the impact of genetic drift cannot be completely discarded, these findings further imply the prominent role of distinct selective pressures and/or ecological and phenotypic plasticity in the population's rapid adaptation to a novel environment.

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Author contribution statement RVD, HS, and LVL originally formulated the idea. AH and AŠ provided samples and sampling permits. LVL analysed the data with input from HS. RVD and LVL wrote the first draft. All authors revised the draft and contributed to manuscript revision.

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Data and code availability All raw sequencing data of island *P. siculus* are available on NCBI and SRA under BioProject ID PRJNA1225591 (released upon publication).

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Permit ID for genetic samples from Sušac, Kopište, Pod Kopište, and Pod Mrčaru (2023): UP/I-352-04/23-08/60, Croatian Ministry of Economy and Sustainable Development. Permit ID for genetic samples from Bijelac (2016): UP/I-612-07/16-48/116, Croatian Ministry of Environmental Protection and Energy. All applicable institutional and/or national guidelines for ethical sampling were followed.

Consent to participate Not applicable.

Consent for publication Not applicable.

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