



Reconstructing the complex colonisation histories of lizards across Mediterranean archipelagos

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Funding information

Fundação para a Ciência e a Tecnologia; Ministero dell'Università e della Ricerca; Mohamed bin Zayed Species Conservation Fund

Abstract

Aim: The Mediterranean Basin is a global biodiversity hotspot and has one of the longest histories of human–biota interactions. Islands host a large fraction of Mediterranean diversity and endemism, but the relative importance of natural versus human-mediated colonisation processes in shaping the distribution and genetic structure of Mediterranean island fauna remains poorly understood. Here, we combine population genomics, demographic models and palaeoshoreline reconstructions to establish the island-colonisation dynamics of wall lizards in Mediterranean archipelagos.

Location: Four Mediterranean archipelagos in Italy and Croatia.

Taxon: The wall lizard *Podarcis siculus*.

Methods: We used ddRAD sequencing to genotype 140 lizards from 23 island and mainland populations. Analyses of admixture and site frequency spectra were used to reconstruct population structure, demographic history and variation of gene flow through time. Genomic results were integrated with palaeogeographical reconstructions and were compared to archaeological evidence of human presence on these islands.

Results: Although many island populations of this species are assumed to be non-native, we find that many islands were colonised long before any known human settlements (230,000–12,000 years ago). This natural colonisation most likely occurred through land bridges during glacial marine regression or by over-sea rafting. On the other hand, islands distant from the continent were often colonised recently, and some of the estimated island colonisation times match historical records of human arrival. We also determine that long-established island populations generally show lower genetic diversity compared to proximate mainland populations, contrary to recently colonised islands that must have experienced higher rates of post-colonisation gene flow.

Main Conclusion: Our integrated approach provides us with the power to accurately quantify the origin, timing and mode of island colonisation. This framework helps to clarify the biogeographical and evolutionary history of island populations, with important implications for conservation and management of island biodiversity.

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KEYWORDS

ddRAD sequencing, Demographic history, Island biogeography, Overseas dispersal, *Podarcis siculus*, Vicariance, wall lizards

1 | INTRODUCTION

Islands hold a disproportionate amount of the world's biodiversity, and the study of the factors driving the origin and maintenance of island diversity has fuelled the formation and testing of evolutionary, ecological and biogeographical theories (Losos & Ricklefs, 2009). During the last millennia, island biotas have been re-shaped by human-assisted movement of fauna and flora, leading to the establishment of non-native taxa and the extinction of island endemic species. The interplay between long-term natural processes and more recent, less-predictable processes complicates our understanding of current evolutionary and biogeographical patterns (Capinha et al., 2015; Helmus et al., 2014; Losos & Ricklefs, 2009). This is also true at the regional scale within global biodiversity hotspots such as the Mediterranean Region (Ficetola & Padoa-Schioppa, 2009; Silva-Rocha et al., 2019).

The complex geological and climatic history of the Mediterranean Basin have promoted the diversification of island flora and fauna through multiple and complex colonisation, isolation and selection processes taking place across different time scales. Quaternary climatic oscillations have played a key role in structuring the current distribution of island endemics and, consequently, their genetic diversity. Sea-level lowering during glacial periods exposed new areas of land with the formation of bridges that allowed mainland populations to colonise islands. These land bridges eventually disappeared during interglacial periods, limiting migration and promoting divergence of island populations (i.e. vicariance). On the other hand, many islands separated by deep-sea channels have never been physically connected to the continent, yet may host dispersal-limited species, suggesting a role for overseas dispersal (Cowie & Holland, 2006; Salvi et al., 2021). Therefore, the genetic composition of island populations has been shaped by island-specific features and species traits, such as spatial isolation, time-since-isolation and dispersal capacity (Garg et al., 2022; Heaney et al., 2005; Roberts, 2006; Wang et al., 2014).

Islands have not only promoted the differentiation of endemic biotas but have also provided favourable areas for their long-term persistence. While many temperate species underwent range contractions during glacial periods and expansions during interglacial periods (Hewitt, 2000, 2004), there is increasing evidence for glaciation-induced expansions in coastal and island habitats (Bisconti et al., 2011; Canestrelli et al., 2007; Salvi et al., 2014; Senczuk et al., 2019). However, several endemic lineages that have formed and persisted on islands throughout the glacial cycles became extinct during the last millennia, due to the arrival of humans and of non-native species (Helmus et al., 2014; Hudson et al., 2016; Nogué et al., 2021; Silva-Rocha et al., 2019). Such human-assisted movements have disrupted species distributions and patterns of genetic diversity on islands, particularly across the Mediterranean. Most

introductions have occurred in the recent past, but some events extend well into prehistoric times (Crees & Turvey, 2015; Hofman & Rick, 2018; Seebens et al., 2017).

The respective roles of ancient natural colonisation and recent introduction events for explaining the genetic structure and geographical distribution of extant taxa remain poorly understood because reconstructing the evolutionary history of island populations is often challenging. Over the past few decades, mitochondrial DNA (mtDNA) has been routinely used to understand historical biogeography (e.g. Kornilios et al., 2010; Podnar et al., 2005; Salvi et al., 2010). Genetic studies have documented very diverse modalities of island colonisation according to the species and the island, and human-assisted dispersal is often the prevailing hypothesis when insular and mainland populations show weak genetic differentiation both in large isolated islands (e.g. Lebarbenchon et al., 2010; Sherpa et al., 2018) and in near-shore islands (e.g. Marchán et al., 2020; Salerno et al., 2023). However, the limited resolution of mtDNA markers means that we cannot discriminate between colonisation events that took place during Quaternary sea-level regression and more recent, human-mediated introductions. Approaches enabling a representative sampling of genome-wide genetic variability allow a more powerful and refined phylogeographical inference, overcoming the limitations of mtDNA markers (Cutter & Payseur, 2013; Delsuc et al., 2005; McCormack et al., 2013). Such approaches have provided information on taxon diversification on islands (Charles et al., 2018; Jensen et al., 2021; Manthey et al., 2020; Papadopoulou & Knowles, 2017), and island population histories (Blumenfeld et al., 2021; Martin et al., 2021; Rosenthal et al., 2021; Sherpa et al., 2019), but very few studies have attempted to disentangle the role of natural versus human-aided dispersal (McDevitt et al., 2022).

Here, we use population genomics to address the role of sea-level oscillations and the effect of human activities on island-colonisation dynamics. Wall lizards are particularly well-suited models for this purpose. Wall lizards are widespread both in Mediterranean islands and in the adjacent mainland, and have a complex biogeographical pattern, with some insular populations assumed to have colonised islands naturally, and other considered to be introduced by humans (Bonardi et al., 2022; Silva-Rocha et al., 2019). However, the lack of genomic data on these populations results in high uncertainty for their status and complicates the identification of the biogeographical processes determining island biodiversity. Among them, *Podarcis siculus* is native to the Italian Peninsula, Sicily, and the Adriatic Coast, but has established invasive populations worldwide (Capinha et al., 2017; Oliverio et al., 2001; Oskyrko, Sreelatha, Hanke, et al., 2022; Oskyrko, Sreelatha, Silva-Rocha, et al., 2022; Silva-Rocha et al., 2012, 2014), and is recorded from ~300 Mediterranean islands (Bonardi et al., 2022). In some of these islands, the lizard is presumably native, with ancient endemic lineages (Podnar et al., 2005;

Senczuk et al., 2018). Yet in other nearby archipelagos, the species has most likely been introduced by humans and may therefore represent a threat to island endemics (Capula, 2002; Ficetola et al., 2021). Nevertheless, in most cases, its biogeographical status is unknown (Bonardi et al., 2022), which challenges management actions.

Based on a dataset of genome-wide single nucleotide polymorphisms (SNPs), we establish the migration dynamics and population demographic history of *P. siculus* in four Mediterranean archipelagos. First, we elucidate whether island populations originate from the proximate mainland, as previously suggested (Podnar et al., 2005; Senczuk et al., 2017), and infer the timescale and the dynamics (founder effects, gene flow) of the colonisation events. Subsequently, estimates of colonisation time are combined with a time-series of sea-shoreline reconstructions and compared to archaeological data. Data of human activity on Mediterranean islands (Martinelli et al., 2021; Paschou et al., 2014; Presti et al., 2019; Rowley-Conwy et al., 2013; Zazzo et al., 2015; Zeder, 2008) suggest that ancient colonisation events (>12 kya) are not compatible with human-assisted introduction and therefore reflect natural colonisation events, possibly favoured by marine regression during ice ages. For more recent colonisation events (<12 kya), the potential joint effects of natural and human-aided dispersal complicate expectations because natural colonisation could have occurred at any time and human introductions enable dispersal across a wide range

of overseas distances. Our reconstruction of colonisation histories of Mediterranean archipelagos by *P. siculus* reveals a complex series of independent colonisation events, either by natural or human-assisted dispersal. Because we clarify the native versus non-native status of island populations (Figure 1), we also discuss the importance of our findings for conservation strategies.

2 | MATERIALS AND METHODS

2.1 | Sampling areas

We collected samples of *Podarcis siculus* in 2014–2015 from 23 localities in four regions; Tuscany: three mainland populations and three islands in the Tuscan archipelago; Dalmatia: two mainland populations, and two northern and three southern islands in the Dalmatian archipelago; Western Sicily: two populations in Sicily and three islands in the Aegadian archipelago; Eastern Sicily: one population in Sicily and four islands in the Aeolian archipelago (Figure 1, Table S1). In each locality, we sampled six to eight lizards. The tail tip (~2 cm) was collected and stored in 95%–100% ethanol before immediately releasing the specimen. The removal of tail tips results in a very low disturbance (see García-Muñoz et al., 2011), as lizards quickly regenerate their tails. All collected samples followed national regulations.

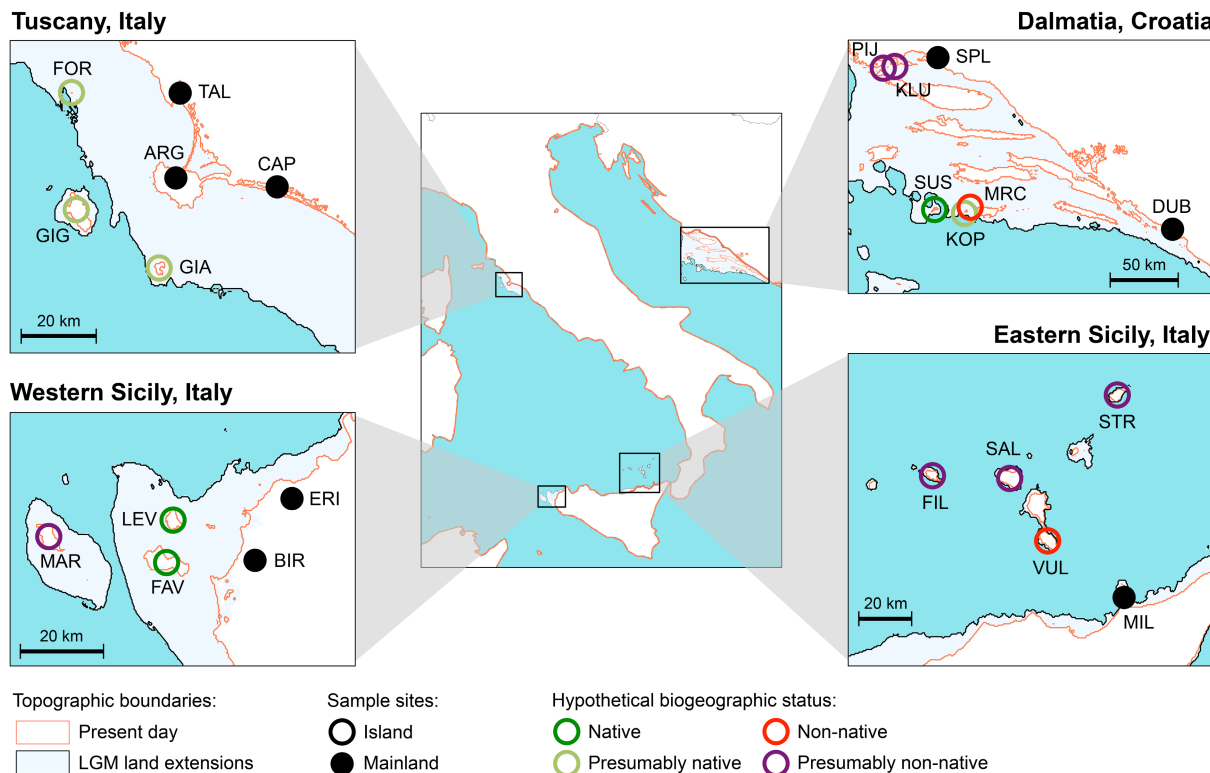


FIGURE 1 Sampling localities within the four geographical regions. The maps show the location of the four geographical regions and the 23 sampling localities: Tuscany (ARG, CAP, TAL) and the Tuscan archipelago (FOR, GIA, GIG); Dalmatia (SPL, DUB) and the Dalmatian archipelago: Sušac (SUS), northern islands (CIN = KLU + PIJ) and southern islands (CIS = KOP + MRC); Western Sicily (BIR, ERI) and the Aegadian archipelago (FAV, LEV, MAR); Eastern Sicily (MIL) and the Aeolian archipelago (FIL, SAL, STR, VUL). The colour of circles indicate the biogeographic status hypothesized by the literature. The map of each region include present-day and Last Glacial Maximum (LGM) shoreline configurations.

2.2 | DNA extraction, library preparation and SNP identification

Genomic DNA was extracted using the DNEasy Blood & Tissue kit. A double-digest restriction-site associated DNA (ddRADseq) experiment was conducted on 150 lizards following the protocol described in Capblancq et al. (2015) with a few modifications (Appendix S1). The identification of SNPs was performed using a de novo approach in Stacks v2.59 (Rochette et al., 2019). Downstream processing of reads including sequence clean-up and loci reconstruction is detailed in Appendix S1. We generated a global dataset including all individuals ($N=140$), and regional datasets for each region: Tuscany ($N=48$), Dalmatia ($N=34$), Western Sicily ($N=28$) and Eastern Sicily ($N=30$). Loci and SNP filtering included minimum coverage, excess of heterozygosity, proportion of missing data, physical linkage and minor allele frequency (Appendix S1; Tables S2 and S3).

2.3 | Population structure and genetic diversity

We first investigated genetic variation with all populations together using the global dataset. We performed a principal component analysis (PCA), calculated population differentiation (pairwise F_{ST}), and inferred population structure using ADMIXTURE (Alexander et al., 2009). We then inferred population structure within each region, repeating these analyses using the four regional datasets. For ADMIXTURE, we tested K possible genetic clusters (range 1–10) and determined the 'optimal' number of K that minimises the 10-fold cross-validation error. As only considering this 'optimal' value would produce an incomplete view of differentiation across populations, we also explored the pattern of population structure for increasing values of K (Janes et al., 2017). PCA and pairwise F_{ST} calculations were performed in R (R Core Team, 2017) using the packages adegenet v2.1.1 (Jombart, 2008) and hierfstat 0.04–22 (Goudet, 2005) respectively. The hierfstat package was used to calculate genetic diversity indices: individual and population observed heterozygosity (H_o), population expected heterozygosity (H_e), allelic richness (AR), inbreeding coefficient (F_{IS}) and population-specific F_{ST} (local drift). Individual H_o was used to test the difference in genetic diversity among islands and between island and mainland populations using ANOVAs in R.

2.4 | Multi-population trees

To reconstruct genetic relationships and identify migration edges (m) between populations, we built a population admixture graph for each region using TreeMix v1.13 (Pickrell & Pritchard, 2012). The program *plink2treemix.py* (<https://bitbucket.org/nygcresearch/treemix>) was used for formatting. We ran 100 independent analyses for each m value ranging from $m=0$ to $m=6$ for Tuscany (four populations), and from $m=0$ to $m=10$ for Dalmatia, Western Sicily, and Eastern Sicily (five populations). The SNP block size for estimating the covariance matrix was set to 100. To determine the optimal

number of m , we stopped adding migration edges when the model explained 99.8% of the total variance (Pickrell & Pritchard, 2012). Models that reached this threshold with $m > 2$ were retested using the R package OptM v0.1.6 (Fitak, 2021). The changes in variance explained by the models were estimated using the ad hoc Δm statistic. After identifying the optimal m , we performed 500 new independent runs with this value and 100-SNP blocks. We retained the tree with the highest likelihood as the best population tree. Bootstrap supports for each node were estimated based on the consensus tree of the 500 sampled trees using the program PHYLIP Consense v3.695 (Felsenstein, 2005) and TreeMix results were visualised using the R package BITE v1.2.0008 (Milanesi et al., 2017).

2.5 | Population demographic models

Population demographic histories were reconstructed for each region separately from the site frequency spectrum (SFS). We generated SFS files of one-population (1D-SFS) and multi-population (joint-SFS) dimensions using easySFS (<https://github.com/isaacovercast/easySFS>). We used the full (folded) SFSs to maintain maximum resolution of the data. One-population demographic inferences were performed using a 1D-SFS as input to Stairway Plot v2 (Liu & Fu, 2020). The proportion of sites used in model training was 67% and the random break points were different for each population, depending on the number of haploid copies (e.g. 3, 6, 9 and 12 for a population of $n=6$ individuals) following the authors' recommendation. We assumed a mutation rate of 1.0×10^{-8} , following rates used or estimated for lizards and other reptiles (Bouzid et al., 2022; Bergeron et al., 2023), and a generation time of 2 years. Each model was based on 100 bootstraps analyses.

Multi-population models were performed using joint-SFS and fastsimcoal v2.7 (Excoffier et al., 2021). The models were designed according to the results of TreeMix and Stairway Plot analyses (Appendix S1; Table S4). We compared four models of divergence: (1) strict isolation, (2) strict isolation and N_e change before divergence, (3) isolation with migration and (4) a combination of models 2 and 3. We performed 100 runs for each model, each run with 40 optimisation cycles that consisted of 100,000 simulations. Support for the different models was evaluated with the Akaike Information Criterion (AIC). We also compared their maximum likelihood (ML) distributions by performing 100 runs of 100,000 simulations using the best-run ML parameters. Model selection might be biased due to linkage (Excoffier et al., 2013; but see Chattopadhyay et al., 2019). However, in our case, the alternative scenarios do not test for changes in topology but only an increase in model complexity. Furthermore, founder effects and gene flow were not imposed, as founder N_e can be as large as current N_e and migration rates can be close to zero (Table S4). The accuracy of the best parameter estimates was evaluated by sampling 50 new bootstrapped SFS to calculate 95% confidence intervals (CIs) and the same procedure for parameter estimation. Estimates of split time (in years) and N_e (in diploid gene copies) were used to calculate the intensity of founder effect, population growth, and the total migration rate per year since isolation (Appendix S1).

2.6 | Overseas distance between islands and source populations

To evaluate the relationship between time-since-isolation and geographical distance at the time of island colonisation, we combined genetic, topographic and sea level data. Topographic data were obtained from the EMODnet Digital Terrain Model (resolution: 1/16 arc-minutes, approx. 115 m) (EMODnet Bathymetry Consortium, 2020) and sea level changes were retrieved from published data (Waelbroeck et al., 2002). For each island-source pair, we created a geographical configuration time-series based on relative sea level and sea level CIs, at each available date between the present-day and 400 kya (above sea level: 0; below sea level: 1). Raster data manipulation was performed using the R packages *sp* v1.3–1 (Hijmans, 2018) and *raster* v2.8–4 (Pebesma & Bivand, 2005). Island-source minimum overseas distances were calculated using the *gdistance* v1.2–2 R package (van Etten, 2018).

We performed a weighted least squares regression between the estimated island-source divergence time and the estimated island-source minimum overseas distance at this time. Weighted least squares regression, contrary to ordinary least squares, does not assume equal variance among observations, and incorporates weights for each observation based on their uncertainty, giving more weight to the most robust observations. This approach thus helped to integrate the knowledge of sea level uncertainties into the regression analysis (Strutz, 2016). Specifically, observations were weighted using the inverse of variance calculated from the overseas distances based on sea level CIs (Waelbroeck et al., 2002). When the variance was zero, we used the minimum variance between other island-source pairs. When two island populations most likely originated from a single colonisation event, we used the mean of the two island-source minimum overseas distances for estimates based on relative sea level, and the minimum and maximum of the two island-source minimum overseas distances for estimates based on sea level CIs. Analysis was performed in R and overseas distances and divergence times were log-transformed.

3 | RESULTS

3.1 | Genomic variation of populations across Mediterranean archipelagos

3.1.1 | Global population structure

Our ddRADseq panel generated ~135 million reads for a total 140 lizards from 23 island and mainland populations across four archipelagos in the Mediterranean: Tuscany, Dalmatia, Western Sicily and Eastern Sicily (Figure 1; Tables S1 and S2). De novo variant calling resulted in 4115 SNPs with a mean coverage of 19X and 19% missing data per sample on average (Table S3). Global genetic differentiation among all populations was strong ($F_{ST}=0.72$). Assessment of population structure using PCA revealed three genetic groups differentiated

by the two first axes (57% of total genetic variance) corresponding to three geographical regions: Tuscany, Dalmatia and Sicily (Figure S1). Analysis using ADMIXTURE revealed an optimal number of genetic clusters was $K=5$ genetic groups (Figure S2), differentiating Western Sicily from Eastern Sicily, Dalmatia (with many admixed individuals), and two clusters in Tuscany with substantial admixture in one of the island localities (GIA; Figure 2). Pairwise F_{ST} ranged between 0.23 and 0.90 between populations of different regions and between 0.00 and 0.77 between populations of the same region (Figure S3).

3.1.2 | Population structure and genetic diversity within archipelagos

Next, we investigated genetic variation within each archipelago: Tuscany (4562 SNPs); Dalmatia (2046 SNPs); Western Sicily (2716 SNPs) and Eastern Sicily (3245 SNPs) (full details in Tables S2 and S3). PCA and ADMIXTURE revealed substructure within all archipelagos (Figures 2, S4 and S5). For Tuscany, the three islands were strongly differentiated from the mainland, with the optimal $K=4$. For Dalmatia, two genetic groups were identified (optimal $K=2$), the first corresponding to the southern islands of the archipelago (SUS, CIS) and the second to the mainland populations (DUB, SPL), with northern islands showing evidence of admixture (CIN) (Figures 2 and S4). In Western and Eastern Sicily, although the optimal K was $K=1$ (Figure S5), it was evident from higher K values and the PCA that all the islands were differentiated from each other and from the mainland (Figures 2 and S4). For Western Sicily, the islands LEV and FAV clustered together, and MAR with Sicily (BIR and ERI). In Eastern Sicily, the islands SAL and FIL clustered together, and the islands STR and VUL clustered with Sicily (MIL).

Island populations had either higher, equivalent or lower genetic diversity than mainland groups (Table S5). Nested ANOVAs revealed higher observed heterozygosity (H_o) in mainland Tuscany than in Tuscan islands ($F_{1,4}=14.48$, $p=0.019$), in mainland Dalmatia and northern Dalmatian islands (CIN) than in southern islands (CIS) ($F_{2,4}=41.37$, $p=0.002$), but H_o was not different between Western Sicily and Aegadian islands ($F_{1,3}=0.49$, $p=0.531$) and between Eastern Sicily and Aeolian islands ($F_{1,3}=1.42$, $p=0.319$; Figure S6). Exploring the genetic diversity within each archipelago revealed marked differences in observed and expected (H_e) heterozygosity, allelic richness (AR) and population-specific F_{ST} values (Table S5). The majority of F_{IS} values were close to 0. H_o showed significant differences among the Tuscan ($F_{4,42}=24.14$, $p<0.001$), the Aegadian ($F_{3,23}=5.99$, $p=0.008$) and the Aeolian ($F_{3,24}=8.66$, $p<0.001$) islands, but not within northern and southern Dalmatian islands ($F_{4,27}=0.28$, $p=0.891$; Figure S6).

3.2 | Colonisation history of islands

3.2.1 | Demographic inference

Based on the strong genetic differentiation observed (Figures 2, S1 and S3), the colonisation dynamics of each archipelago were

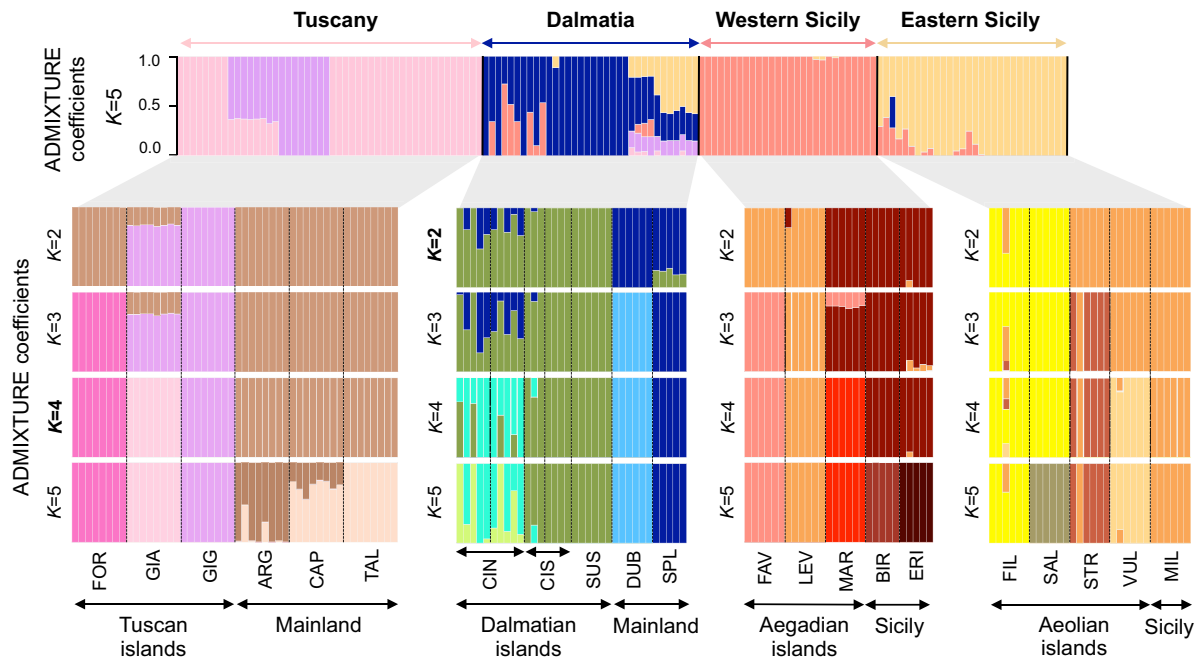


FIGURE 2 Population genetic structure. ADMIXTURE ancestry coefficients for $K=5$ and all 140 individuals (top, global dataset) and for each region (bottom, regional datasets). Optimal number of genetic clusters: global, $K=5$; regional (in bold): Tuscany, $K=4$; Dalmatia, $K=2$; Western Sicily, $K=1$ and Eastern Sicily, $K=1$ (Figures S2 and S5).

reconstructed separately, using the topology of TreeMix best population trees that revealed a best number of migration events of one within Tuscany, Dalmatia and Western Sicily, and two within Eastern Sicily (Figure 3, S7 and S8). Current effective population sizes (N_e) inferred using Stairway Plot analyses and migration events identified by TreeMix were used to calibrate demographic models (Appendix S1). Fastsimcoal demographic inference of the timescales and modes of divergence showed that the most complex model, including founder effects and gene flow, was the best model for all the regions (lowest Δ -likelihood and AIC, non-overlapping ML distributions; Table S6, Figures S9 and S10).

In Tuscany, the N_e of mainland populations started to increase around 180 kya, followed by the expansion of the GIG island population around 100 kya (Figure 4), and the divergence of GIG and GIA from the mainland occurred around 65 (31–100) kya (Figure 5). This was most likely a single event with a low founder N_e , followed by a rapid genetic subdivision (63 (56–71) kya; Figures 5 and 6). Effective migration rates suggest gene flow from mainland Tuscany to GIA after the initial divergence, while GIG remained genetically isolated, in accordance with our ADMIXTURE results (Figures 2, 6 and S11). The population inhabiting the tiny islet of Formica Grande (FOR) diverged more recently (0–10 kya), which was accompanied by a founder effect before isolation, followed by a strong bottleneck (Figures 4–6). This population has the lowest current N_e and H_e among the three islands (Figure S6), despite evidence for post-divergence gene flow with GIG (Figures 2, 3 and 5).

In Dalmatia, mainland populations showed an expansion after 140 kya followed by constant high N_e (Figure 4). For Dalmatian islands, CIN showed an apparent decrease of N_e , while SUS and

CIS showed small but constant N_e over time. The most distant island population (SUS) diverged in strict isolation from the mainland population around 230 (180–280) kya (Figure 5). The two mainland populations SPL and DUB diverged around 25 (19–32) kya, and the northern (CIN) and southern (CIS) Dalmatian islands diverged from the SUS population at 14 (12–15) and 0.6 (0.2–1.0) kya respectively. Consistent gene flow between SUS and CIS, and relatively high founder N_e , maintained the level of diversity in CIS (constant N_e ; Figures 4–6). Similarly, gene flow between mainland Dalmatia (SPL) and CIN explains that many individuals in CIN are admixed and show the highest H_o in the Dalmatian region (Figures 2–6, S6 and S11).

In Western Sicily, all populations showed very consistent demographic histories, with an expansion between 50 and 20 kya, followed by constant N_e over time (Figure 4). The two island populations LEV and FAV originated from a large ancestral population (high founder N_e) that diverged from Western Sicily around 34 (20–49) kya and that split into LEV and FAV around 13 (11–15) kya (Figures 5 and 6). This was accompanied by a strong decline in N_e , especially in LEV that diverged in strict isolation, whereas FAV remained connected to Sicily by gene flow (Figure 6). The furthest island population, MAR, diverged from Sicily around 10 (9–11) kya (Figure 5). High migration rates between FAV and MAR after their respective divergence from Sicily contributed to an increase in N_e and explains the admixture pattern we found in MAR (Figures 2–6, and S6). We also found more recent post-colonisation gene flow between LEV and mainland Sicily (<1.2 kya; Figures 3, 5 and S11).

In Eastern Sicily, the population MIL and two island populations (VUL and STR) showed a demographic expansion between 50 and

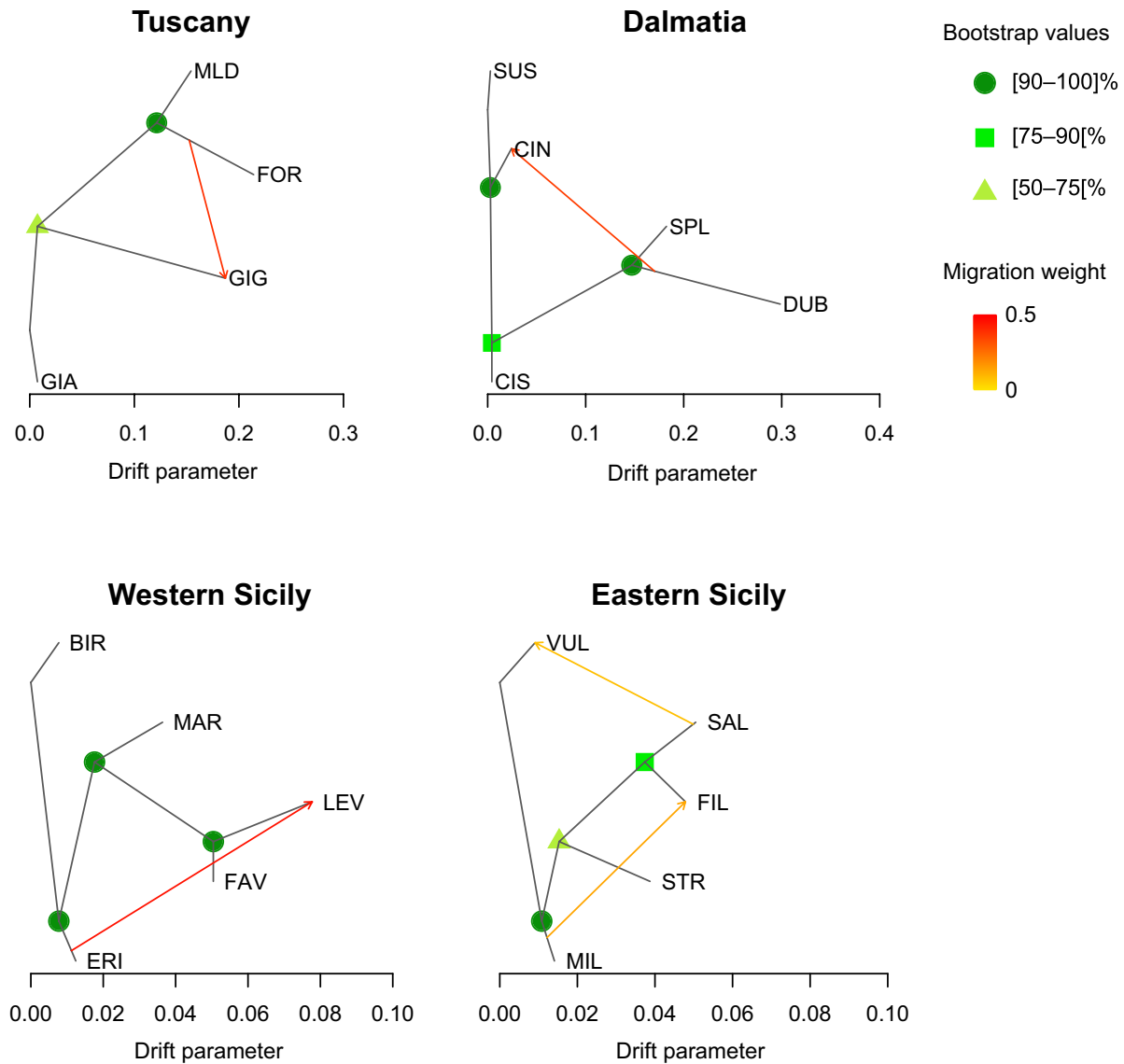


FIGURE 3 Multi-population trees. The number of migration edges (m) between populations was determined from 100 independent TreeMix analyses. The best tree topology was identified from 500 new independent TreeMix runs with the best m value. Bootstrap supports for each node were estimated based on the consensus tree of the 500 sampled trees and are represented by green circles (90%–100% bootstrap support), green squares (75%–90% bootstrap support) and green triangles (50%–75% bootstrap support). The migration weight is represented by colour intensity, as shown in the figure.

20 kya, whereas SAL and FIL show a distinct history and originated from an ancestral population that diverged from MIL around 29 (25–34) kya (Figures 4 and 5). This ancestral population then split into FIL and SAL around 11 (10–12) kya, which was accompanied by a decrease in N_e in both populations (Figures 5 and 6). The population SAL diverged in strict isolation from the mainland, whereas FIL remained connected to Sicily by gene flow until 1 kya although evidence for a single admixed individual suggests more recent gene flow in FIL (Figures 2–6). The two other island populations (STR and VUL) diverged more recently, 4.8 (4.3–5.3) and 0.9 (0.5–1.4) kya respectively. The STR population experienced a strong founder effect and diverged in strict isolation, although our ADMIXTURE

results show evidence of a first-generation migrant from the mainland (Figure 2). The population colonising VUL was highly diverse and subsequently connected by gene flow with SAL (Figures 2–6, and S11).

3.2.2 | Relationship between time-since-isolation and sea-crossing distances

The minimum overseas distance between islands and their respective source has been strongly influenced by changes in sea level over the past 350 kya (Figure S12). Some islands were likely fully

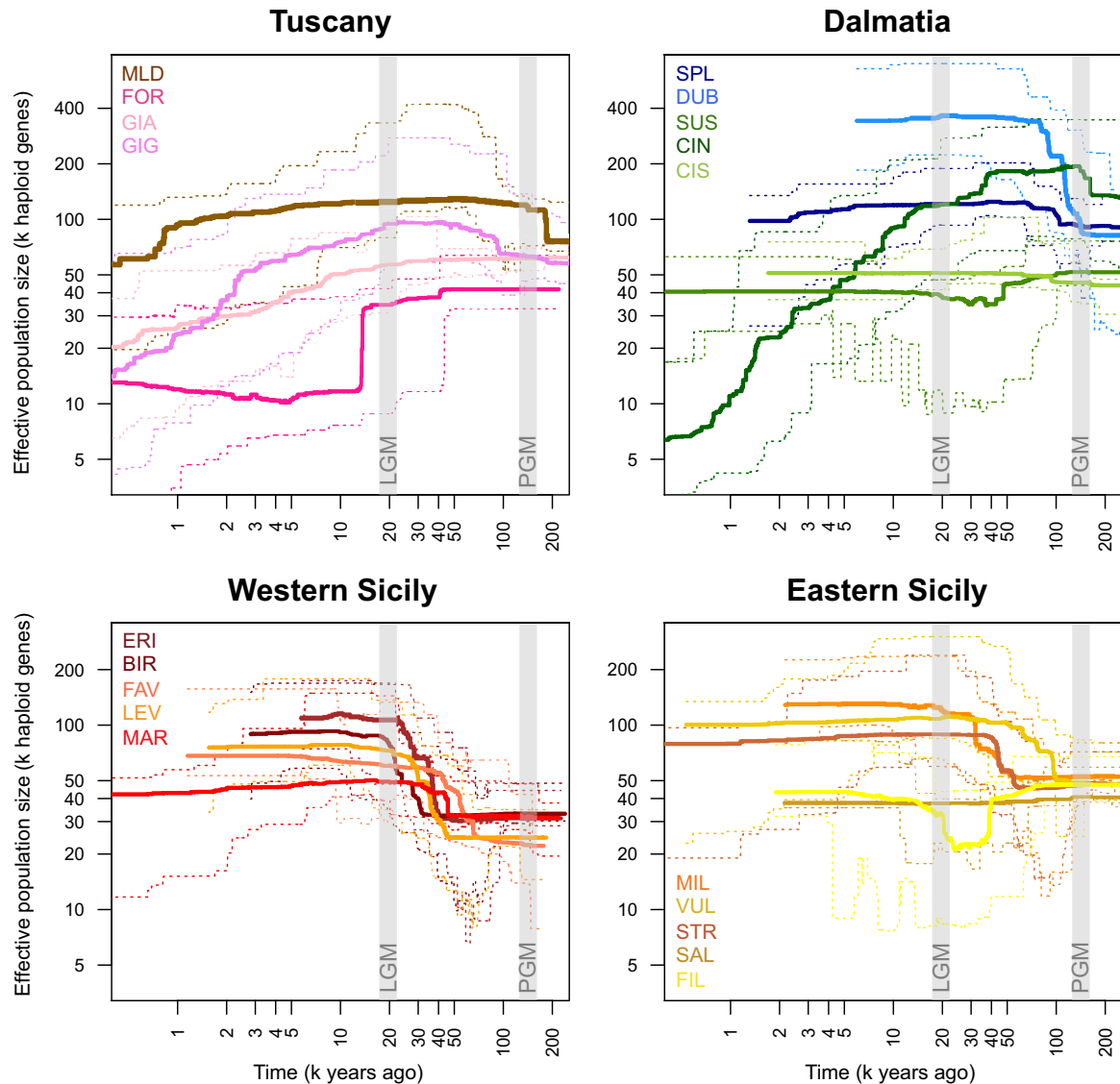


FIGURE 4 Changes in effective population size (N_e) over time. Solid lines represent the median and dotted lines the 95% CIs generated from 100 bootstrap replicates using Stairway Plot. Vertical grey boxes denote the Last Glacial Maximum (LGM; ~20 kya) and the Penultimate Glacial Maximum (PGM; ~140 kya).

connected to the mainland for long time periods during glaciations (~100,000 years during the last glaciation; Western Sicily: FAV and LEV) or occasionally during glacial maxima (Tuscany: GIG and FOR; Dalmatia: SUS, CIN and CIS). Other islands were never connected to the mainland, but the overseas distance was greatly reduced: 4 km between Tuscany and GIG and 2 km between Western Sicily and MAR. On the contrary, the four Aeolian islands have never been connected to Eastern Sicily, and the overseas distance remained large (>20 km) even during glacial maxima (Figure S12). The minimum overseas distances at estimated dates of divergence ranged between 0 and 56 km (Figure 7). We found a positive relationship between time-since-isolation (divergence time in years) and overseas distances between islands and their source populations at this time (linear model: $R^2=0.33$, $F_{1,8}=5.40$, $p=0.049$; Figure 7).

4 | DISCUSSION

4.1 | Population genomics, palaeogeographical and archaeological data inform island biogeographical patterns

Quaternary climatic and palaeogeographical events are often pinpointed as the major drivers of present-day biogeographical patterns in the Mediterranean Region (Blondel, 2010; Hewitt, 2011). Nevertheless, we still have an incomplete view of the interplay between these keystone events and prehistoric human movements in shaping Mediterranean insular biodiversity. The pattern is particularly intricate for near-shore islands, because their geographical proximity with the continent induced episodic permeability to natural dispersal and facilitated early colonisation through human

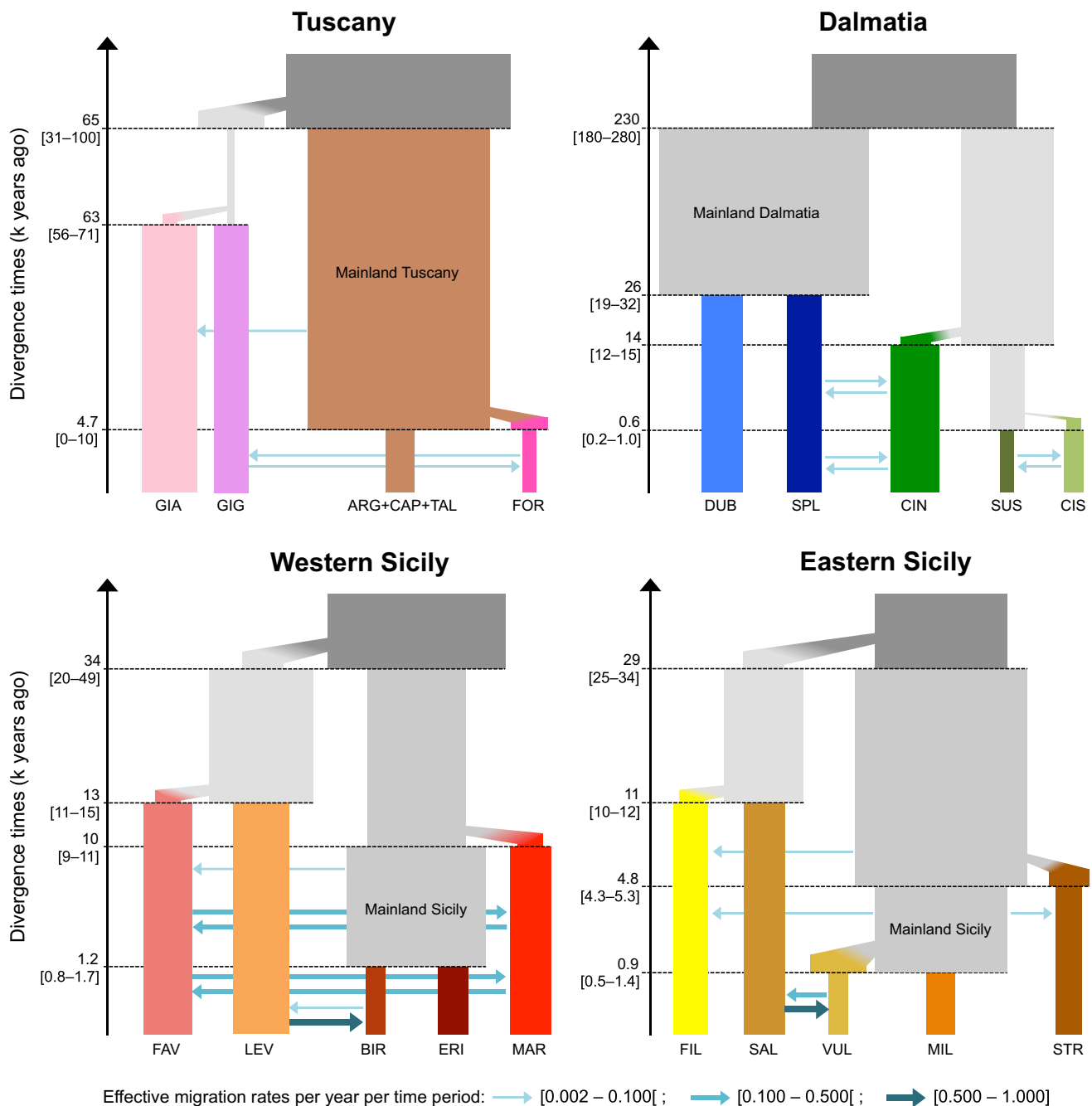


FIGURE 5 Divergence models for the four Mediterranean archipelagos. The best model for all archipelagos included founder effects and gene flow. Effective population sizes (N_e) are provided in Table S4. For effective migration rates, only rates ≥ 0.002 are represented (Figure S11). Divergence times correspond to best ML parameter estimates, and their 95% CIs from 50 bootstrap parameter estimates.

intervention. Differentiating between natural and human-aided dispersal processes generally involves the evaluation of two main criteria: the time-since-isolation and contemporary geographical isolation (Essl et al., 2018; Wilson et al., 2009). By combining high-resolution genomic data and palaeogeographical data, our analyses revealed that the current distribution of wall lizards across Mediterranean islands is the result of a complex dispersal framework, determined by biogeographical affinities (dispersal from the adjacent continent/islands during marine regressions) and multiple

overseas colonisation events, either by natural or human-mediated dispersal.

Island-source divergence times revealed that seven islands were colonised long before known human settlements (>12 kya). The number of propagules and their frequency of arrival are expected to decrease in more isolated islands, but remote islands may be prone to establishment of populations into previously vacant niches (Whittaker & Fernández-Palacios, 2007). Our assessment of colonisation patterns confirms that ancient colonisation events

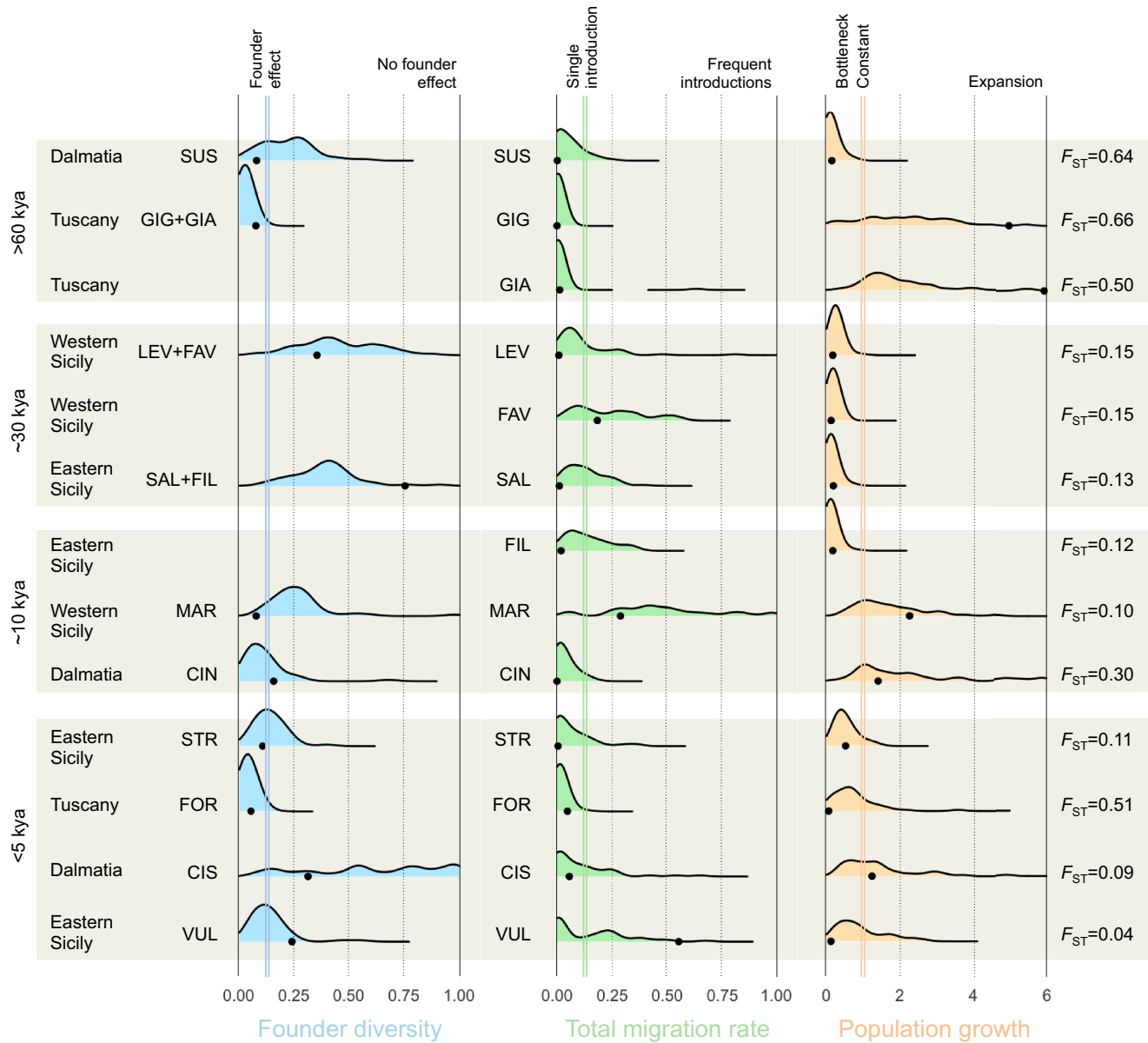


FIGURE 6 Island-colonisation dynamics across Mediterranean archipelagos. Founder diversity, total migration rate per year since isolation and population growth were calculated from fastsimcoal ML parameter estimates (see Appendix S1). Black points: best model estimates; density distributions: bootstrap estimates. F_{ST} : genetic differentiation between island and source populations (see Figure 5 for sources), mean F_{ST} if several possible sources (Figure S3).

occurred when islands were connected or closer to the continent (Figure 7), supporting the hypothesis that natural colonisation is most likely when island isolation is minimised. The presence of vast emerged areas at the end of the last glacial period probably allowed the expansion of the Western Sicily ancestral population into the islands of LEV and FAV, whereas the subsequent rise of sea level rapidly disconnected these islands from one another (Presti et al., 2019). This result supports the hypothesis that glaciation-induced expansions are a main driver of the distribution of island biotas in the Mediterranean Basin (Bisconti et al., 2011; Podnar et al., 2004; Salvi et al., 2014; Senczuk et al., 2019). Similarly, a continental population colonised the Tuscan islands GIA and GIG

thanks to reduced overseas distances (~10km) during the last glacial period. This narrow distance also facilitated colonisation by other species, as shown by faunistic similarities between these two islands (Fattorini, 2010a). Sea-level lowering may have also promoted the colonisation of the Dalmatian island SUS. However, possible secondary contact between Dalmatian and Sicilian lineages makes it difficult to propose a scenario for Dalmatian islands without additional data covering both sides of the Adriatic coast and other Dalmatian islands (Figure 2; Podnar et al., 2005).

The distant volcanic islands of the Aeolian archipelago (Eastern Sicily) were never connected to Sicily, but genomic and archaeological data support the hypothesis of natural dispersal for two islands

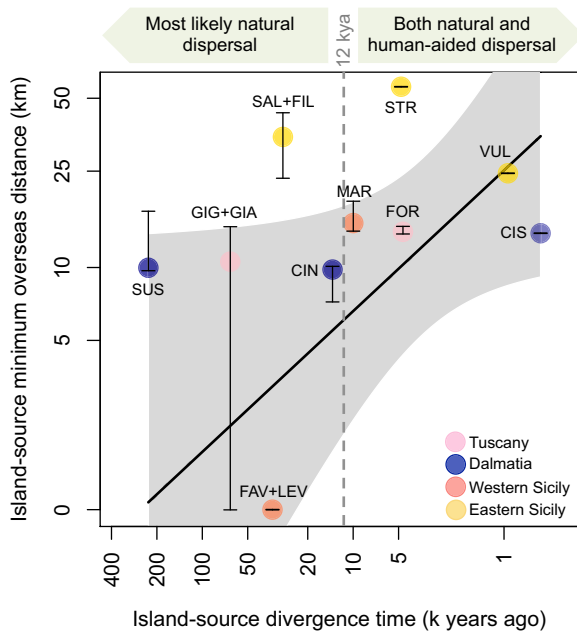


FIGURE 7 Relationship between island-source divergence time and overseas distance at time of isolation. Vertical error bars represent the minimum overseas distance confidence intervals at each estimated divergence time, used in model weighting to account for differing sea level uncertainties across estimated divergence times.

(SAL and FIL), as the estimated divergence times (10–34) are much older than any human settlement in these islands (7–7.5 kya; Martinelli et al., 2021). This ancient colonisation is likely related to lizard ability to cross the sea over long distances (20–45 km), which has occasionally been shown (Escoriza, 2021; Glor et al., 2005; Welt & Raxworthy, 2022). This was probably facilitated by island-to-island colonisation events and by natural drifting on sea surface currents directed towards the Aeolian archipelago from Eastern Sicily (Escoriza, 2021; Fattorini, 2010b; Stroschio et al., 2011), and supports the hypothesis that transmarine dispersal events between landmasses and islands have played a major role in biotic exchange across the Mediterranean, and in the diversification of many terrestrial vertebrates including lizards (Hewitt, 2011; Mendes et al., 2017; Salvi et al., 2021).

Most populations in anciently colonised islands diverged in strict isolation, with the sea acting as a strong barrier to gene flow. Interestingly, we still found evidence for subsequent gene flow with the mainland in GIA, probably associated with LGM land connections forming after the original colonisation event (Figure S12). Consistent with other studies (Lourenço et al., 2018; Wang et al., 2014), long-established populations tend to show lower genetic diversity and higher differentiation compared to mainland populations. These patterns of distribution, diversity and differentiation highlight the key role of natural dispersal and vicariant events for the biogeography of the Mediterranean Basin.

Recent colonisation events (<12 kya) mostly happened in islands further from the continent (Figure 7). This is probably because

human transport loosened barriers to dispersal, even though long-distance natural dispersal remains possible. For three of the recent colonisation events, the time-since-isolation perfectly matches the known colonisation history of islands by humans, strongly supporting the role of human-assisted introductions. The colonisation of Marettimo (MAR) from Western Sicily (9–11 kya) overlaps with the first dated evidence of human presence in this island (9–13.5 kya; Presti et al., 2019), and might be even more recent than estimated considering the limited sampling of putative sources in Sicily. Phylogeographical studies on other taxa showed strong genetic similarities between populations from Western Sicily and the two islands LEV and FAV, and endemic lineages occurring on MAR (Fiorentino et al., 2008; Senczuk et al., 2019). On the contrary, *P. siculus* populations in Western Sicily are genetically closer to the MAR population, supporting the hypothesis of a more recent, human-aided introduction. In Eastern Sicily, Stromboli (STR) has a history of strong volcanic activity, and humans probably colonised this island relatively recently (4–5.5 kya; Orlando et al., 2018; Martinelli et al., 2021), which matches our inferred date of arrival of *P. siculus* in this island (4.3–5.3 kya). Also due to volcanic activity, Vulcano (VUL) is the only Aeolian island without prehistoric settlements, but evidence of mining and agriculture during the Middle Ages (Manni et al., 2019) suggests that humans may have facilitated an introduction of *P. siculus* that is estimated at 0.5–1.4 kya. These results confirm a strong interaction between past human societies and Mediterranean insular biodiversity (Gippoliti & Amori, 2006; Médail, 2022). We also found a tendency of increased dispersal distances and an increased gene flow between populations in recent times (Figure 5 and 7), supporting the rising impact of human activity on species redistribution and genetic homogenisation (Capinha et al., 2015; Olden et al., 2004). This might be particularly important for anthropophilic species like wall lizards, which have been accidentally introduced through the transportation of materials and cultivated plants (Santos et al., 2019; Silva-Rocha et al., 2015, 2019).

The congruencies between genetics and geological/archaeological data were striking, confirming the power of genomic approaches for detecting major events determining the demography and distribution of species. However, natural and human-aided dispersal events are not always easy to tease apart. For instance, the actual drivers for the colonisation of islets (FOR, CIN, CIS) cannot be identified, as short overseas distances and multiple sources are compatible with both dispersal processes. Furthermore, other processes besides dispersal probably contributed to the observed patterns. First, recently colonised islands host populations with varying levels of genetic diversity because each of them has its own demographic history (founder diversity, time-since-isolation, post-colonisation gene flow), which makes it challenging to generalise about patterns of genetic diversity across islands (García-Verdugo et al., 2015). Second, recent colonisation of the islands by *P. siculus* does not exclude a more ancient presence. Demographic stochasticity might have exacerbated the extinction risk of small populations due to bottleneck effects (FOR that was connected to mainland during the LGM). Our results also suggest a combined

effect of environmental stochasticity and human colonisation history. The volcanic activity in the Aeolians could have reduced suitability of some islands (VUL, STR) that were recolonised more recently, while the species persisted in other islands (SAL, FIL) within the same archipelagos.

4.2 | Population genomics for a better management of island biodiversity

The introduction of non-native species is the main cause of biodiversity decline in islands (Bellard et al., 2017; Nogué et al., 2021), making the detection of non-native species critical. Low-density genetic markers have limited resolution for accurate estimates of population parameters, with the consequent risk of defining inappropriate management actions (Supple & Shapiro, 2018). We demonstrate that population genomic approaches enable us to clarify when (colonisation time approximated by divergence time), and how (natural dispersal vs. human transportation), populations established on an island, thus determining the status of species (native vs. non-native) when it is uncertain.

Until now, it has been assumed that the wall lizard is non-native in many Mediterranean islands (Bonardi et al., 2022), yet we detected at least seven island populations that established following old colonisation events (>20 kya) and are not compatible with human-aided introductions. The joint effect of these natural and human-aided processes within each archipelago has major consequences for management. For instance, the Aeolian archipelago hosts the Critically Endangered, endemic lizard *Podarcis raffonei*. The decline of this species is often explained by the recent introduction of *P. siculus* (Capula, 2002; Ficetola et al., 2021), but our reconstruction of colonisation history suggests a more complex pattern with both natural and human-aided colonisation events. Only in some islands it is likely that *P. siculus* has been introduced recently by human activities (e.g. Vulcano), thus supporting the importance of control actions (on *P. siculus*) for the preservation of endemic species.

Whether populations should be considered as native or non-native depends on the criteria used to classify them. Time-since-isolation is a criterion generally used to define the biogeographical status of a population (Essl et al., 2018), but it remains complicated for human-aided introductions occurring a few millennia ago nearby the native range (Crees & Turvey, 2015). Furthermore, demographic inferences have their own limitations and aspects of uncertainty, including genetic resolution for model selection and parameter estimation (Excoffier et al., 2013) and the set of a priori values, which can subsequently impact parameter estimation. Uncertainty also arises from the conversion of estimated parameters into absolute values, depending on the reliability on mutation rate and generation time, which are notoriously difficult to estimate in non-model species.

Finally, the relationship between divergence time and colonisation time can be complex. Divergence and colonisation times might be close when colonisation involves a single dispersal event from a small fraction of the mainland population. However, if islands remained

connected to the mainland for long periods, the period between colonisation and divergence can be relatively long, thus divergence time can underestimate the true colonisation time. In other cases, the colonisation can be recent but the divergence time overestimated, for example, if the ancestral source population has not been sampled or become extinct (Stroschio et al., 2011), if secondary contact between previously isolated lineages erase part of the past history (as might have occurred in mainland Dalmatia), or if the model does not account for ancient population expansion (Momigliano et al., 2021). Nevertheless, the fact that divergence times match extremely well with the known human colonisation or geological history for most of colonisation events supports the robustness of our conclusions.

5 | CONCLUSIONS

Resolving the interplay between natural and human-aided dispersal processes in the colonisation of islands is pivotal to identify the processes determining biodiversity in insular regions. Like many widespread Mediterranean species, the Italian wall lizard shows an extraordinarily large intraspecific diversity and a strong phylogeographical structure. By integrating fine-grained genomic and palaeogeographical data, we obtained precise reconstructions of the tempo and dynamics of arrival of island populations, and post-colonisation gene flow. Furthermore, archaeological information helped in teasing apart the effects of biogeographical processes during the Pleistocene versus human-aided introductions during the Holocene. Such integrated approach is invaluable for understanding the factors and processes promoting species colonisation and diversity, and can be applied to the reconstruction of biogeographical history, biological invasions and natural range expansions, helping to identify appropriate management strategies for native/non-native island species.

ACKNOWLEDGEMENTS

We thank Delphine Rioux (LECA, UMR UGA-USMB-CNRS, Grenoble, France) for help with the DNA laboratory work. Alex Cortada and Joana Mendes assisted in the field sampling in Croatia. This study was funded by the Italian Ministry for Research (PRIN project 'Hybrid' - 2017KLZ3MA to DS and GFF; and the program 'Rita Levi Montalcini' to DS) and by the Mohamed bin Zayed Species Conservation Fund (Project 162514415). MAC and ISR are supported by grant 28014 02/SAICT/2017 and SFRH/BD/95745/2013 from FCT, Portugal. Lizards were captured and handled under permits from the Italian Ministry of Environment (PNM-2012-0009747 and MiTE-2022-0024156) and Croatian authorities (UP/I-612-07/15-48/117).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Individual ddRAD sequences (demultiplexed paired end fastq files) are available under project accession number PRJEB62841 at the

European Nucleotide Archive repository (<http://www.ebi.ac.uk/ena>), and sample accession numbers are provided in Supporting Information Supplementary Table S2. The SNP datasets and fastsimcoal2 files used for analyses are available at Dryad Digital repository (<https://doi.org/10.5061/dryad.7h44j100f>).

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BIOSKETCH

Stéphanie Sherpa is an early career researcher at the University of Milan (DESP). Her research focuses on the mechanisms shaping the genetic composition and geographical distribution of animal populations, and how the evolution of these population parameters relates to historical biogeographical processes, species evolutionary history and recent processes in the context of global changes. This research work was part of her post-doc and initiated as part of the PhD thesis of I. Silva-Rocha supervised by D. Salvi, M.A. Carretero and G.F. Ficetola in the framework of a collaborative project aiming at understanding the underlying

processes of Mediterranean reptile's biodiversity at both broad and local scale.

Author Contributions: Daniele Salvi, Gentile Francesco Ficetola and Miguel Angel Carretero designed the study. Iolanda Silva-Rocha, Daniele Salvi, Miguel Angel Carretero and Gentile Francesco Ficetola performed the sampling. Iolanda Silva-Rocha and Thibaut Capblancq performed molecular biology work. Stéphanie Sherpa designed the analytical workflow and performed the analyses with contributions from Josephine R. Paris, Daniele Salvi, Iolanda Silva-Rocha, Thibaut Capblancq and Gentile Francesco Ficetola. Stéphanie Sherpa wrote the manuscript with substantial contribution from Daniele Salvi, Thibaut Capblancq, Josephine R. Paris and Gentile Francesco Ficetola. All authors have contributed to and approved the final version of the manuscript.

SUPPORTING INFORMATION

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

How to cite this article: Sherpa, S., Salvi, D., Silva-Rocha, I., Capblancq, T., Paris, J. R., Carretero, M. A., & Ficetola, G. F. (2023). Reconstructing the complex colonisation histories of lizards across Mediterranean archipelagos. *Journal of Biogeography*, 00, 1–16. <https://doi.org/10.1111/jbi.14739>