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Discrete Isolation and Continuous Area Drive Genetic Divergence Across Mediterranean Islands

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ABSTRACT

Aim: Comparison between islands and equivalent mainland areas to dissect the effect of area, isolation and species traits in determining island genetic endemicity and genetic differentiation.

Location: The Western Mediterranean region.

Time Period: Current.

Major Taxa Studied: Butterflies (Lepidoptera, Papilionoidea).

Methods: We analysed 10,367 COI sequences from 105 species, 34 islands and 47 sea straits, along with four functional species traits. We compared determinants of genetic diversity (nucleotide diversity, *NucDiv*), endemic mutations (EM) and the *Dst* fixation index between island populations and similarly sized mainland populations (Continental Area Equivalents, CAEs). Generalised linear mixed models tested fixed effects and interactions of island characteristics and species traits. We also evaluated whether population-level effects (individual species increasing differentiation with island size and isolation) or community-level effects (larger, less isolated islands hosting more genetically divergent species) better explain observed island patterns.

Results: CAEs and islands exhibited highly distinct genetic signatures. *NucDiv* was higher in CAEs while *Dst* was higher across sea straits than between land areas separating CAEs, particularly for larger islands and non-migratory species. EM increased with island area, especially in non-migratory and small species. Island communities were significantly nested. Widespread species also showed lower genetic variation, thus producing a strong community-level effect. The population-level effects showed a weaker effect. Counterintuitively, increasing isolation did not increase divergence, as both processes tended to reduce genetic differentiation in more isolated islands.

Main Conclusions: Comparing islands with CAEs reveals that even narrow sea barriers of a few kilometres greatly reduce gene flow in non-migratory butterflies, increasing genetic differentiation to a greater extent than similar mainland distances. After controlling for the presence of sea straits, island size predicts genetic divergence while increasing isolation

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does not. Overall, endemicity remains low, indicating that mainland-driven genetic turnover, rather than in situ evolution, dominates insular genetic patterns.

1 | Introduction

Islands host a distinctive portion of global biodiversity, attracting the attention of conservationists and evolutionary biologists (Warren et al. 2015; Whittaker et al. 2017; Fernández-Palacios et al. 2021). Classical biogeography identifies island isolation, area and species traits as key factors shaping insular biotas, influencing both species assemblages and genetic divergence of island populations relative to their mainland counterparts (Lomolino 2000; Martín-Devasa et al. 2024). In general, small size and strong isolation reduce immigration and colonisation while increasing extinction, resulting in species-poor and genetically-depauperate communities (Lomolino 2016; De Kort et al. 2021; Matthews et al. 2021). Because isolation acts as a filter, insular communities exhibit an over-representation of generalist and dispersive taxa. This leads to a compositional bias (disharmony), where taxonomic and functional proportions of island biotas deviate significantly from those of their mainland source (Lomolino 2016). At the same time, islands are centres of endemism, produced by both anagenesis and cladogenesis (Warren et al. 2015), contributing disproportionately to global biodiversity (Lomolino 2016; Whittaker et al. 2017).

While the role of isolation and area in shaping species composition is well established, their influence on intraspecific genetic variation between island and mainland populations remains less explored (Heaney et al. 2005; Leigh et al. 2021). Distance from continents is a major driver of differentiation, as limited gene flow promotes allopatric divergence (Heaney et al. 2005; Whittaker and Fernández-Palacios 2007; Kisel and Barraclough 2010). Island area may also matter: larger islands host larger, more stable populations, which may facilitate persistence of lineages and promote long-term divergence (Warren et al. 2015; Matthews et al. 2016). Accordingly, minimum island sizes for endemic species have been identified across taxa (Diamond 1977; Coyne and Price 2000). Area and isolation can also interact: sea barriers limit colonisation, while large islands support higher carrying capacities. This generates a numerical advantage for early colonisers, creating a long-lasting 'priority effect' (Orsini et al. 2013; McCulloch and Waters 2019). Once established, these large populations become resistant to genetic decay by gene exchange, as the massive number of residents buffers the gene pool against introgression from occasional immigrants. This process promotes relictuality and allows ancestral lineages to persist for thousands of generations, whereas on smaller islands frequent extinction-recolonisation events tend to periodically reset genetic variation (McCulloch and Waters 2019; Scalercio et al. 2020; Arif et al. 2021). Beyond island characteristics, species traits modulate the potential for genetic divergence: highly vagile species tend to colonise islands more frequently, increasing gene flow and reducing divergence (Burney and Brumfield 2009; Lomolino 2016; Gálvez-Reyes et al. 2021; Suárez et al. 2022).

The possible correlation between island features and genetic divergence may arise from two non-exclusive processes. On one hand, larger and more isolated islands may promote higher divergence by providing stable environments and stronger barriers to gene flow for any occupying taxa (the population-level process). On the other hand, island features may act as filters that shape community composition; larger and less isolated islands host a higher proportion of specialist and poorly-dispersive taxa, which are inherently more prone to differentiation (the community-assembly process) (Dennis et al. 2012; Bastidas-Urrutia et al. 2025). Disentangling these two processes is essential to understanding whether genetic variation is driven by island geography or by intrinsic features of the species they host.

The effects of area, distance and species traits on genetic variation are not unique to islands. On the mainland, differentiation also generally correlates with geographic distance (i.e., isolation by distance) and species traits (Oleksa et al. 2013; Dapporto et al. 2024; Sucháčková Bartoňová et al. 2024). A promising framework for such comparisons is continental area equivalents (CAEs), where randomly selected mainland areas of similar size and sampling effort serve as benchmarks for evaluating specific features of island biotas (Jamoneau et al. 2022). However, direct comparisons of geographic distance effects on genetic differentiation in island versus mainland populations remain rare due to limited genetic data for species-rich taxa across multiple islands.

Over the past two decades, the mitochondrial cytochrome c oxidase subunit I (COI) gene has become a standard marker for animal DNA barcoding (Hebert et al. 2003). While mitochondrial markers have limitations (Galtier et al. 2009), COI has been pivotal for macrogenetics, enabling broad-scale assessments of genetic variation across taxa and regions (Leigh et al. 2021; French et al. 2023; Dapporto et al. 2024) and linking species' functional traits to genetic structure and matrilineal dispersal (Burney and Brumfield 2009; Fujisawa et al. 2015; Dapporto et al. 2024). This potential has been renewed by metabarcoding, which allows large-scale biodiversity inventories on islands (e.g., Noguerales et al. 2023).

Here, we exploit the unique case of Western Mediterranean butterflies (Lepidoptera, Papilionoidea), a large and diverse group with available COI data at unprecedented spatial and taxonomic resolution. We assembled a comprehensive dataset of 105 species, 34 islands and 10,367 COI sequences, to:

1. Assess the contributions of island geography and species traits to genetic variation. We model how island area, isolation (strait length) and species functional traits (e.g., dispersal ability, ecological specialisation) interact to shape genetic differentiation and diversity. These patterns are further evaluated against CAEs to determine whether the observed genetic structure is a specific consequence of insular geography or mirrors general scaling effects of area and distance found on the mainland.
2. Disentangle the mechanisms underlying the relationship between island area and isolation and genetic variation.

Specifically, we separate two non-exclusive processes: (i) a community-level (compositional) process, whereby island features filter which species are present, thereby influencing the genetic divergence of island assemblages; (ii) a population-level (within-species) process, whereby intraspecific genetic divergence varies with island features within species. These two processes were analysed separately and then compared in terms of their contribution to the observed patterns.

2 | Material and Methods

2.1 | COI Sequences and Island Data

We obtained COI data for Western Mediterranean butterflies from the Integrated and Open Butterfly Database (*iodatabase*). Additionally, 216 sequences were generated for this study (Supporting Information) and are publicly available in the MEDIS (204) and BDE (12) datasets in the Barcode of Life Data System (BOLD). We compiled a list of all Western Mediterranean islands with butterfly COI sequences and their surface area (km^2). Relevant sea straits were identified as follows:

i. the narrowest strait length (d) separating the focal island from the nearest larger landmass (island or mainland; Figure 1).

ii. additional straits within a $2d$ radius, selecting in each case the strait connecting the focal island to the largest of the surrounding landmasses.

We also included the straits wider than $2d$ of Corsica-Italian mainland, Sicily-North Africa and Elba-Corsica, due to their biogeographic relevance for butterfly faunas (Vodă et al. 2015; Dapporto et al. 2024).

2.2 | Specimen Selection and Genetic Differentiation Index

For each strait, we selected sequences from the island or landmass and within 100 km of either strait endpoint. Three exceptions were made for Sardinia, Corsica and Sicily since the main mountain areas hosting most of the species are located about 160 km far from the straits. A species was included for a given strait only if represented by at least five specimens, with a minimum of two on each side.

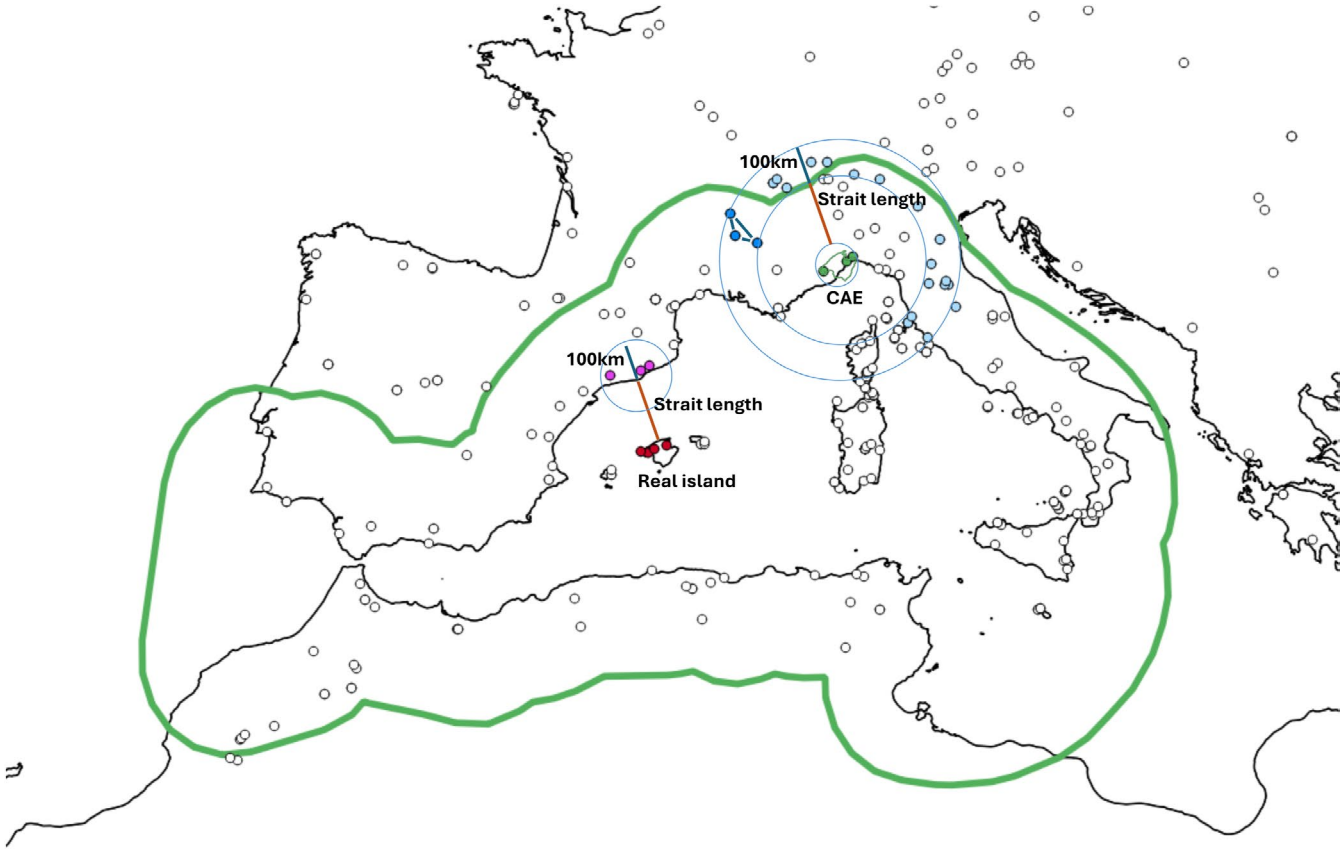

FIGURE 1 | Example of the procedure used to select populations for one island (Mallorca) and for one continental area equivalent (CAE). Specimens for a given species are represented as dots. Island specimens (in red) were compared to mainland reference specimens (in purple), selected within an area of 200 km diameter around the closest mainland area. Specimens from CAEs (in green) were selected within a 250 km buffer from the Mediterranean coast (green line). Within each CAE, potential mainland reference specimens (in pale blue) were identified to match the same strait length and, among them, a random group exhibiting similar mutual distances (200 km max) were finally selected as mainland references for the CAE (in dark blue).

For each species-strait, we computed a dissimilarity matrix of uncorrected pairwise distances among specimens (function *dist.dna*, R package *ape*, Table S1.1 for main package information). From these we calculated the *Dst* fixation index between the two sides of each strait (Meirmans and Hedrick 2011):

$$Dst = \mathrm{HT} - \mathrm{HS}$$

where HT is the average p-distance among all sequences, and HS is the average p-distance within population on each strait side. *Dst* quantifies absolute genetic differentiation, ranging from negative values (indicating higher intra-population than overall differentiation) to the maximum p-distance; negative values were set to zero (Meirmans and Hedrick 2011).

2.3 | Nucleotide Diversity and Endemic Mutation Scoring

For each species in each island we calculated nucleotide diversity and endemic mutations (Dapporto et al. 2024). Nucleotide diversity was calculated using the *nuc.div* function of the *pegas* R package as the mean pairwise p-distance among sequences within each island. To calculate endemic mutations we first identified single-island endemic haplotypes by comparing island COI sequences with available datasets in BOLD for the Western Palearctic, Asia and North America. The divergence of endemic haplotypes was quantified as the sum of minimum distances between each endemic haplotype and its closest relative in the haplotype network. As the number of endemic haplotypes does not increase linearly with sample size, we standardised endemic mutations by the square root of the number of sequenced specimens (EM, function *endemic.mutation, GLQ* R library).

2.4 | Comparison With Continental Area Equivalents

To compare genetic differentiation across sea straits with similar mainland areas, we applied the 'continental area equivalents' (CAEs) approach (Jamoneau et al. 2022). We calculated the maximum distance between two points within each island (MD), corresponding to the maximum possible distance between two samples. For the largest islands in the study area, Sardinia and Sicily, this distance was approximately 250 km. Accordingly, mainland reference specimens were those within a 250 km buffer from the Mediterranean coast (Figure 1). For species in each island, we searched for groups of mainland specimens with mutual distances shorter than MD by producing a hierarchical clustering (average linkage) based on a geographic distance matrix and cutting the dendrogram at a height equal to MD.

Among these groups, representing potential CAE populations with a spatial extent comparable to islands, we randomly selected one among the closest in sample size to the focal island population. Using these specimens, we calculated *NucDiv* and EM. For *Dst* comparisons across straits, CAE specimens of the focal island were selected as above; reference mainland specimens from the larger area were chosen at distances of $MD/2 + d$ and $MD/2 + d + 100$ km (or 160 km) from the selected CAE (Figure 1). Hierarchical clustering based on geographic distances was cut at 200 km (or 320 km) to define reference CAE populations of larger landmasses (Figure 1). These groups were randomly ordered, and we retained the first one meeting at least five specimens overall, with at least two on each side of the virtual strait. If no suitable group was found, we moved to the next MD-based CAE until a valid CAE was eventually identified. We then calculated *Dst* among these specimens. For endemic species from islands surrounding Sardinia and Corsica, we used each of these two large islands separately to define CAEs. To avoid mixing biogeographically distinct regions, samples from North Africa and Southern Europe were treated separately. If both regions provided valid CAEs for a given species, one was chosen at random.

2.5 | Species Traits

We considered four butterfly functional traits: (1) wing index, a continuous measure of wing size; (2) phenology, derived as the first component from a Principal Component Analysis including flight period length, number of generations per year and the first and last month of flight; (3) number of larval host plant genera (Middleton-Welling et al. 2020); and (4) migratory behaviour, coded as a binary categorical variable. The 11 species known for obligate or facultative migratory tendencies were classified as migratory, whereas all remaining species were classified as non-migratory. Migratory species were identified based on literature data: *Colias crocea*, *Pontia daplidice*, *Pontia edusa*, *Pieris rapae*, *Pieris brassicae*, *Lampides boeticus*, *Leptotes pirithous*, *Vanessa cardui*, *Vanessa atalanta*, *Issoria lathonia* and *Danaus chrysippus*.

2.6 | Data Analysis

2.6.1 | Objective 1

To investigate the degree of genetic differentiation and endemic haplotype divergence between island and CAEs and their correlation with species traits and strait features, we fitted Generalised Linear Mixed Models (GLMMs, R package *glmmTMB*). Potential confounding effects due to collinearity among island features and species traits were assessed via a Pearson correlation matrix. We used as response variables in separate models: *Dst*, *NucDiv* and EM. The random effect structure was adapted to each response variable: for *Dst* we included strait identity as a random factor because a single island may involve multiple straits. For *NucDiv* and EM we used island identity as the random intercept and the shortest strait as an isolation measure. To account for phylogenetic non-independence, we included species identity nested within the genus as an additional random effect. Area of the focal island, strait length and the four butterfly traits were used as predictors together with the number of sequences available for each species–island or species-strait combination to account for differences in sampling effort. Island area, strait length and the number of host plants used were log-transformed. Predictors were scaled and centred to facilitate comparison of effect sizes. All models used Tweedie distribution due to skewed distribution responses.

We fitted three GLMMs for each genetic index to assess the role of main effects and interactions separately. This approach

allowed us to assess both the direct effects of each variable and their first-order interaction with region type (islands versus CAEs). The second-order interactions between island features, species traits and region type correspond to a fourth quadrant analysis (Brown et al. 2014). Since CAE samples were randomly selected among suitable candidate groups, we repeated the full analysis 100 times and averaged the model results. To account for multiple testing across the three biological response variables (45 *p* values obtained in ANOVA tables), we applied the Benjamini–Hochberg False Discovery Rate (FDR) procedure using the *p.adjust* function of the *stat* R package. This approach was preferred over the overly conservative Bonferroni correction to maintain sufficient statistical power to detect complex ecological interactions while strictly controlling the expected proportion of false positives (Benjamini and Hochberg 1995).

2.6.2 | Objective 2

We tested for community nestedness to determine whether the fauna of species poor islands represent non-random subsets of the richer ones. Occurrence data available in literature (Dapporto et al. 2014) were updated with new records generated in this study. We used the NODF metric (Nestedness metric based on Overlap and Decreasing Fill) which quantifies: Decreasing Fill, ensuring that the number of species decreases from richer to poorer sites; and Paired Overlap, measuring the degree to which the species present in a poorer site are a proper subset of those in a richer site. The average of these values (NODF) ranges from 0 to 100, perfectly nested. The observed NODF was compared against a null distribution from 1000 randomisations, preserving island richness while reshuffling species occurrences (*nestednodf* and *oecosimu* functions in the *vegan* R package).

To investigate the relative contribution of community-level and within-species processes, we used a set of complementary models linking the three indexes of genetic variation to species incidence (the number of islands occupied by a species) and island features. These models capture distinct, but not statistically independent, components of variation. Consequently, the variance explained by each model does not represent a formal partition of total variance, but rather an approximation of the relative strength of the underlying processes.

As a first step, to test whether widespread species show lower genetic variation, we calculated, for each species, the mean genetic variation across all islands (EM and *NucDiv*) or across island–source comparisons (*Dst*), and regressed these values against species incidence.

As a second step, to quantify the overall relationship between island features and genetic variation, we calculated, for each island, the mean value of each genetic index (*Dst*, EM and *NucDiv*) across species and regressed it against island area and minimum strait length. We used the minimum distance per island so that isolation could be treated as an island-level property comparable across species.

In the third step, we quantified the community-level (compositional) process. For each species, we used its mean genetic variation across islands (*Dst*, EM or *NucDiv*) as an intrinsic species-level property. For each island, we calculated the mean of these species-level values for the species occurring on that island based on faunistic data. This index represents the expected genetic variation based solely on species composition, independent of within-species processes. We modelled the relationship between this index and island area and minimum strait length using linear models.

Finally, to assess whether island features affect genetic indexes within species, we fitted Generalised Linear Mixed Models (GLMMs) using glmmTMB, with divergence as response:

$$(\text{Dst or EM or NucDiv}) \sim \log(\text{feature}) + (0 + \log(\text{feature}) \mid \text{Species})$$

where log(feature) represents either log-transformed and standardised island area or minimum distance. This model allows species-specific slopes while excluding species-specific intercepts, so that between-species differences in baseline divergence are not absorbed by the random structure. The strength of the within-species process was quantified using the marginal R^2 of the model. *p* values have been adjusted using FDR.

3 | Results

3.1 | Objective 1

A total of 10,367 COI sequences from 105 species across 34 islands (Figure 2A–G) and continental area equivalents (CAEs) were selected at least once for island communities or in the 100 random selections of CAEs. A correlation among explanatory variables confirmed the independence of our predictors: the correlation between log-transformed distance and log-transformed area was low (0.300), and all correlations among species traits were consistently below 0.400 (Table S1.2), indicating that no confounding effects exist between island features and biological traits.

Nucleotide diversity (*NucDiv*) showed a single significant effect being higher in CAEs than in islands (Figure 3A; Table 1). Haplotypes unique to individual islands occurred in 36.3% of island populations, but only 6.6% consisted exclusively of endemic lineages. In average across the 100 randomisations for CAEs, these proportions were similar (39.9% and 8.9%, respectively). EM was positively correlated with sample size, since EM = 0 was frequent, and even a single endemic haplotype in a large sample was sufficient to yield a non-zero value. Unlike *NucDiv*, CAEs did not exhibit different levels of EM compared to islands (Table 1). A significant first-order interaction between region type (islands versus CAEs) and area (Table 1) indicated that EM significantly increased with area only on islands. An interaction between wing size and region type and a second order interaction between wing size, region type and area revealed that EM are more frequent in large islands and this effect is more pronounced in smaller species (Figure 3D). Similarly, a first and second order interaction of EM with phenology, region type and island area revealed that species characterised by short phenology show a similar increase in EM as area increases, while species with long phenology only show a positive relationship on islands (Figure 3C).

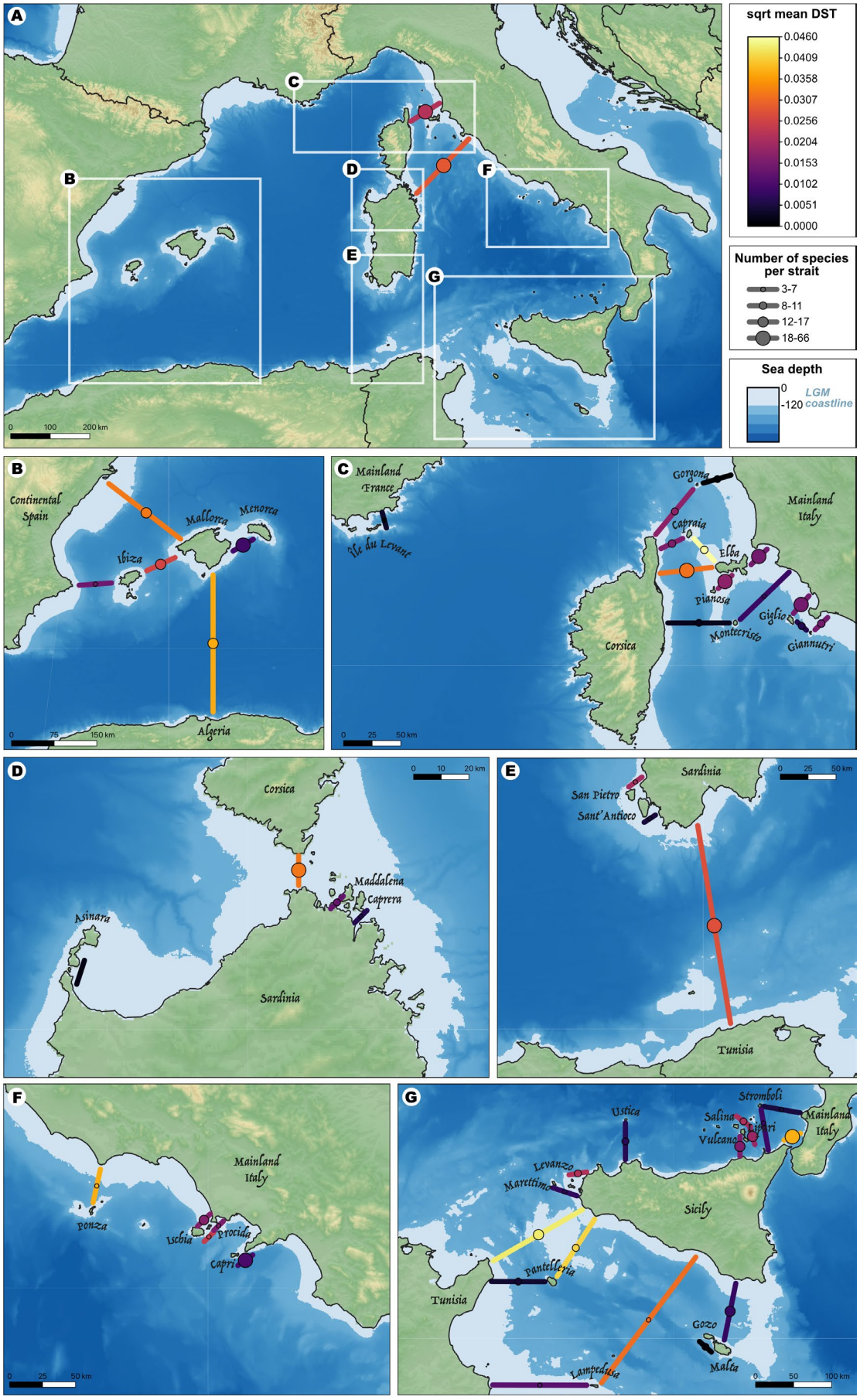

FIGURE 2 | Legend on next page.

FIGURE 2 | A map of the study area (A) and detailed maps of small islands (B–G). The selected straits are indicated with lines and the mean *Dst* (square root transformed) for each strait is shown using a colour gradient (depicted in the legend). The size of the circles represents the number of species involved in each strait divided into four quantiles.

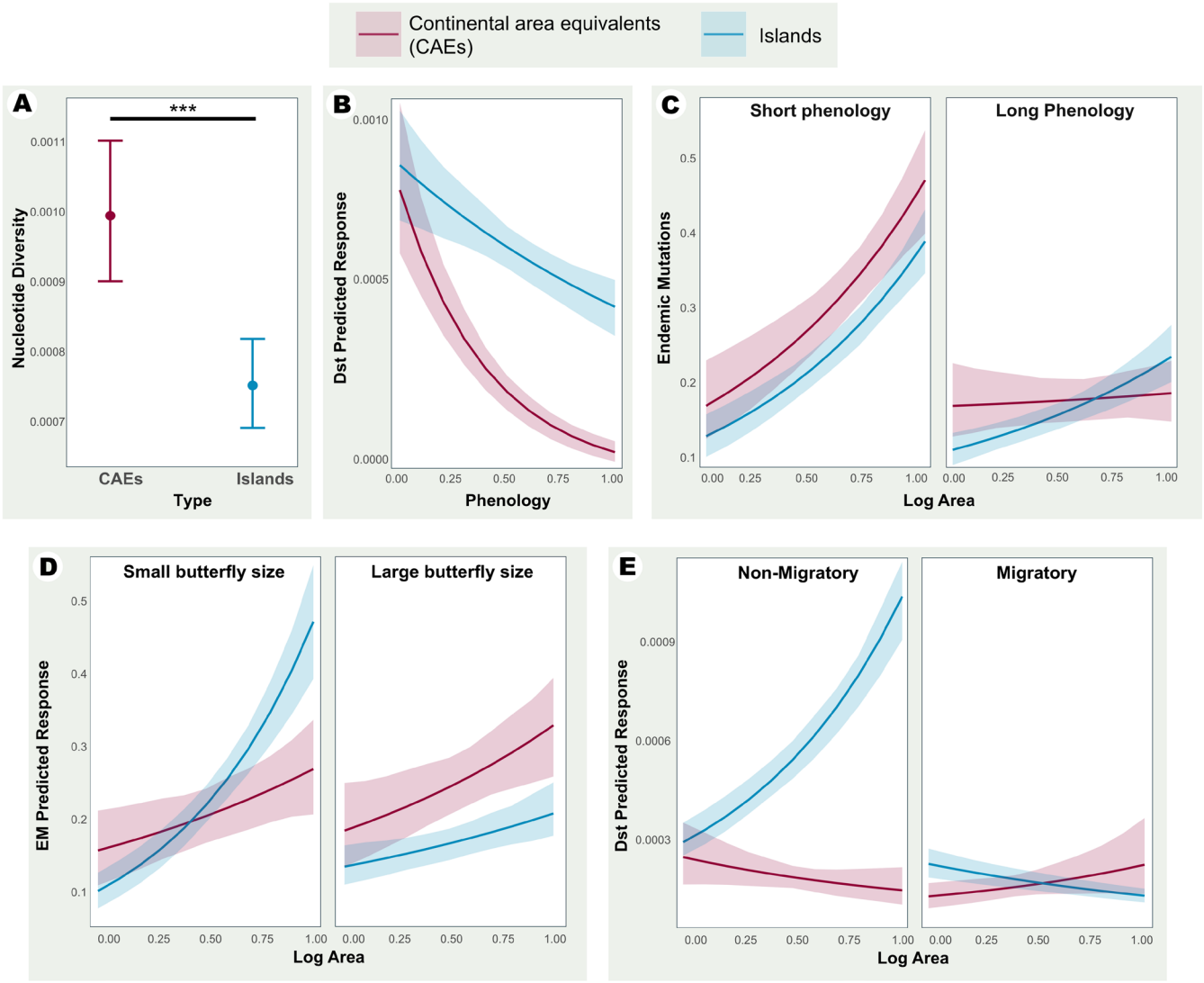

FIGURE 3 | Significant effect determining nucleotide diversity and endemic mutations. (A) The effect of island and CAEs in determining nucleotide diversity (the y axis represents estimated marginal means); (B) the interaction between phenology and islands versus CAEs (region type) determining *Dst*; (C) the second order interaction between area, phenology and region type determining endemic mutations (EM); (D) the second order interaction between area, wing size, region type in determining EM; (E) the second order interaction between island area, migratory behaviour and region type determining *Dst*.

For *Dst*, we analysed 652 species × straits for islands and an average of 663 for CAEs, covering 84 species, 34 islands and 47 straits (Figure 2A–G). Region type, phenology and migratory behaviour showed significant main effects: *Dst* was higher across sea straits and in non-migratory species characterised by short phenologies (Table 1). *Dst* also showed three first-order interactions revealing that the negative correlation between *Dst* and phenology was much steeper in CAEs (Figure 3B). The other two first order interactions of *Dst* combining region type with migratory behaviour and area were encompassed in a single significant second-order interactions: genetic differentiation increased with area, but only in non-migratory species on islands (Figure 3E; Table 1). Overall, region type was involved in 12 significant effects (FDR adjusted $p < 0.05$) out of 45 tested for this variable (Table 1), area in 7 effects out of 18, while strait length did not show any significant effect. Among species traits, significant effects were generated by: migratory behaviour = 4, phenology = 4, wing index = 2, host plants = 0 (Table 1).

TABLE 1 | ANOVA table for the three series of models including i) only the main effects of geographic features of islands and CAEs (logDist, log-transformed strait length; logArea, log-transformed area), species traits (Wing-index, wing size; Host_plants, number of host plant genera; Migrat_behav, migratory behaviour) and membership of island or CAE (type), ii) first interactions between type and traits and iii) second order interactions between type, species traits and island features.

	NucDiv		EM		*Dst*	
	Chsq	**BH_Adj_P**	**Chsq**	**BH_Adj_P**	**Chsq**	**BH_Adj_P**
Sequences	5.395	0.156	**13.454**	**0.010**	1.742	0.447
Type	**21.306**	**0.001**	6.532	0.153	**82.476**	**0.000**
logDist	0.530	0.653	0.462	0.653	6.944	0.105
logArea	0.410	0.670	**18.212**	**0.010**	1.784	0.442
Wing_index	1.902	0.367	0.255	0.710	1.407	0.428
Host_plants	0.739	0.520	1.843	0.367	0.118	0.790
Phenology	1.506	0.400	**17.302**	**0.001**	**7.333**	**0.044**
Migrat_behav	0.147	0.748	0.403	0.650	**9.354**	**0.019**
	Chsq	**BH_Adj_P**	**Chsq**	**BH_Adj_P**	**Chsq**	**BH_Adj_P**
Sequences	5.272	0.170	**8.812**	**0.047**	0.585	0.653
Type:logDist	2.982	0.428	4.023	0.392	7.669	0.133
Type:logArea	1.663	0.636	**29.493**	**0.000**	**15.597**	**0.014**
Type:Wing_index	4.503	0.367	**36.005**	**0.000**	9.808	0.077
Type:Host_plants	9.807	0.105	2.454	0.442	6.375	0.170
Type:Phenology	3.310	0.392	**19.968**	**0.002**	**31.275**	**0.000**
Type:Migrat_behav	7.293	0.170	3.541	0.410	**19.255**	**0.001**
	Chsq	**BH_Adj_P**	**Chsq**	**BH_Adj_P**	**Chsq**	**BH_Adj_P**
Sequences	4.971	0.189	5.573	0.133	0.562	0.653
Type:logArea:Migrat_behav	6.325	0.410	**57.618**	**0.000**	**35.592**	**0.000**
Type:logArea:Wing_index	1.012	0.705	**13.910**	**0.014**	4.579	0.367
Type:logArea:Phenology	4.937	0.367	**11.521**	**0.047**	8.540	0.205
Type:logArea:Host_plants	2.916	0.409	7.270	0.112	7.696	0.113
Type:Migrat_behav:logDist	5.067	0.442	9.067	0.271	5.842	0.442
Type:Wing_index:logDist	1.353	0.653	1.619	0.633	4.481	0.367
Type:Phenology:logDist	1.514	0.650	3.057	0.396	3.382	0.409
Type:Host_plants:logDist	7.674	0.133	3.030	0.413	1.599	0.636

Note: Columns show the results for nucleotide diversity (*NucDiv*), endemic mutations (EM) and *Dst*. Reported *p* values (*p*) have been adjusted for multiple testing across all models and response variables using the Benjamini–Hochberg False Discovery Rate (FDR) procedure. Significant effects are highlighted in bold based on these adjusted values.

3.2 | Objective 2

Species in our island dataset exhibited a significantly nested distribution (NODF = 59.50; mean NODF in R1 null models = 47.382; $p = 0.001$), meaning that poorer communities from small and isolated islands are mostly composed of widespread species. Widespread species exhibited lower *Dst* and EM than range-restricted species (Table 2, Table S1.3), providing the basis for a compositional effect.

In the total effect, island area showed a strong positive relationship with mean *Dst* and EM, indicating that larger islands harbour more genetically differentiated populations (Figure 4A,C). *NucDiv* and strait length did not show any significant effect (Table 2).

For *Dst* and EM, larger islands hosted assemblages composed of intrinsically more divergent species with no effects on *NucDiv* (compositional process, Table 2, Figure 4A,C). In contrast, for

TABLE 2 | Summary of the relationships between island features, species incidence and genetic variation across the three genetic indices (*Dst*, EM and *NucDiv*).

Phenomenon	Incidence	Total effect	Comp.Path	Within-sp.Path
Dst-Area	0.080* (–)	0.238* (+)	0.298** (+)	0.088*** (+)
Dst-Dist	0.108* (–)	0.003 ns	0.171* (–)	0.051* (–)
EM-Area	0.041ns	0.272* (+)	0.232** (+)	0.142*** (+)
EM-Dist	0.043ns	0.002ns	0.161* (–)	0.004ns
NucDiv-Area	0.023ns	0.025ns	0.016ns	0.004ns
NucDiv-Dist	0.020ns	0.025ns	0.135ns	0.001ns

Note: For each combination of genetic index and island feature (area or minimum distance), we report the coefficient of determination (R^2), the direction of the relationship (sign in parentheses), and its statistical significance. The columns represent: (i) The species-level relationship between genetic variation and species incidence (Incidence), (ii) the total island-level effect of the environmental feature (Total effect), (iii) the community-level (compositional) process based on species assemblages (Comp.Path) and (iv) the within-species process estimated from mixed models (Within-sp.Path). R^2 values are not strictly additive, as processes are not statistically independent. Significance codes for FDR adjusted *p*-values: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ns = not significant.

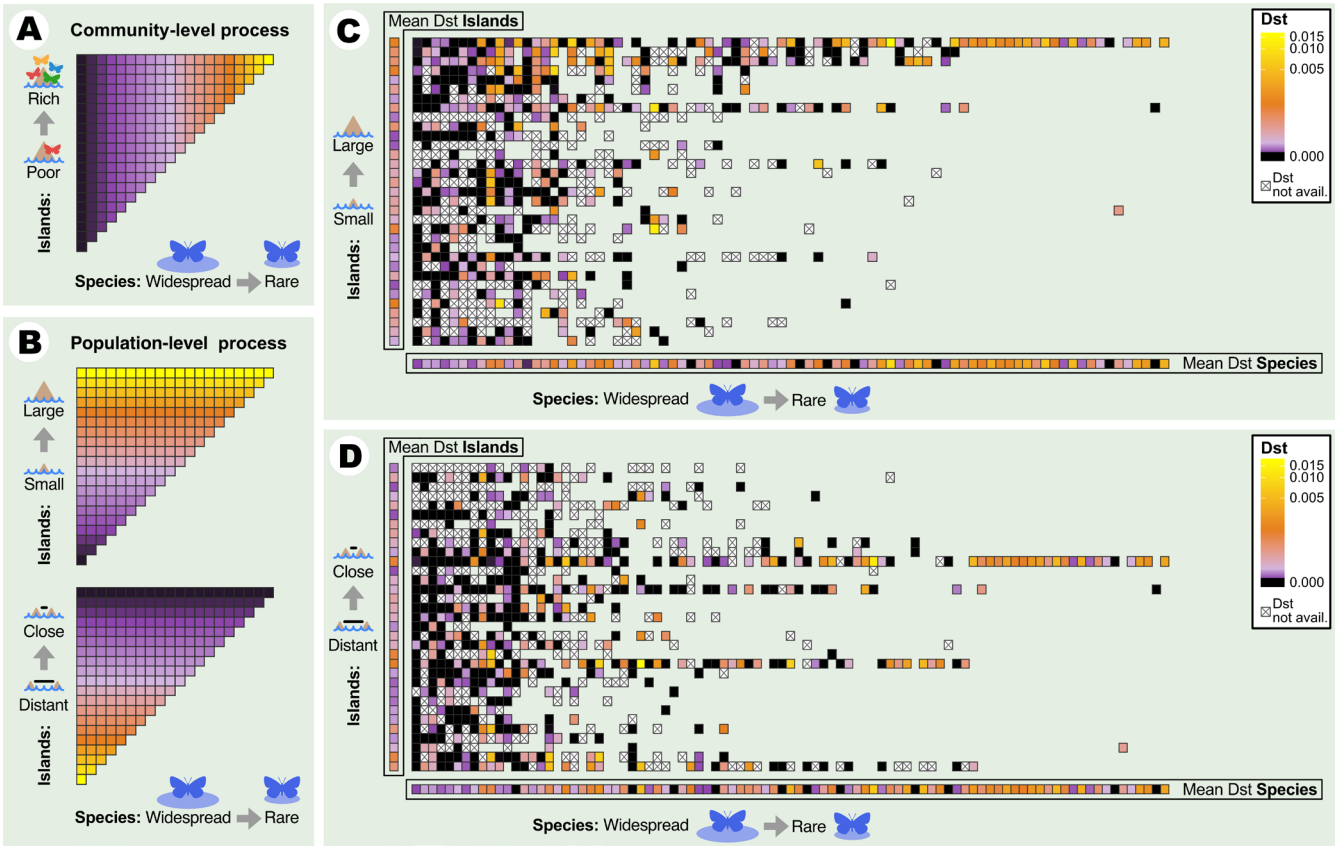

FIGURE 4 | Conceptual and empirical representation of community-level and within-species processes shaping genetic divergence (*Dst*) across islands. (A) Community-level process (hypothesis). Island area acts as a filter on species composition. Because rare species tend to exhibit higher intrinsic divergence, larger islands are expected to harbour assemblages composed of more genetically divergent taxa. (B) Population-level process (hypothesis). Island features affect genetic divergence within species. Larger islands promote higher divergence, whereas isolation (distance) may produce opposing effects depending on gene flow and extinction–colonisation dynamics. (C) Observed pattern for island area. Heatmap of *Dst* values across species (columns) and islands (rows), with species ordered from widespread to rare and islands ordered by increasing area. Colours represent *Dst* values, with warmer colours indicating higher divergence. Grey crossed cells indicate species–island combinations for which *Dst* could not be estimated. (D) Observed pattern for minimum distance. Heatmap of *Dst* values across species and islands ordered by increasing isolation.

minimum distance, the compositional process showed a significant negative relationship for *Dst* and EM (distant islands host assemblages of intrinsically less divergent species, Table 2, Figure 4B,D, Figures S1.1–S1.6).

Within species, genetic divergence (*Dst* and EM) also increased with island area (Table 2, Figure 4A,C). Strait length had a single significant negative effect on *Dst*, revealing that populations separated by shorter straits show higher divergence (Table 2, Figure 4B,D).

For both *Dst* and EM, the variance explained by the community-level models was higher than that explained by the within-species models (Table 2), suggesting a stronger contribution of species composition than of within-species responses to island features. However, these values should not be interpreted as additive components of explained variance because the two processes are not statistically independent and may capture overlapping portions of the same biological signal.

4 | Discussion

By comparing intraspecific genetic variation of butterflies between islands and continental area equivalents (CAEs) in the Western Mediterranean, we found that island populations exhibit distinctive genetic signatures. The presence of any sea strait, captured in the comparison between islands and CAEs, encompassed most of the significant effects, whereas strait length had no additional influence. Island area, in contrast, consistently increased genetic variation. Haplotype diversity was reduced on islands, without detectable effects of island or species traits. Conversely, despite the absence of a clear endemic signature (EM) on islands, genetic differentiation (*Dst*) was higher on islands than in CAEs. Migratory behaviour, large wing size and long adult phenology further modulated differentiation patterns, likely reducing differentiation across sea straits by enhancing gene flow (Objective 1). Finally, our data support the well-established view that species-poor island communities represent nested subsets of richer assemblages. Rare species, restricted to larger islands, exhibit higher values of genetic indices. This compositional effect explains a larger proportion of the increase in genetic divergence than the within-species response to island area. In line with the findings from Objective 1, the hypothesis that more isolated islands should harbour more divergent populations is not supported. Instead, the compositional process revealed that more isolated islands host subsets of species with lower intrinsic divergence and within-species analyses show a weak but consistent negative relationship between isolation and *Dst* as well (Objective 2).

4.1 | Area and Isolation in Determining Genetic Variation

The main source of variation in the three genetic indices is the contrast between islands and CAEs (i.e., the presence of any sea strait), with increasing strait length showing no additional effects. This is unexpected, as many sea straits are narrow (10 of 47 < 5 km). Butterflies are generally regarded as strong dispersers, able to track habitats (Devictor et al. 2012) although dispersal ability varies widely depending on habitat predictability and species ecology. Yet non-migrant species often avoid crossing water bodies (Wood and Samways 1991), drastically reducing gene flow and generating sharp taxonomic and genetic contrasts on either side (Scalercio et al. 2020; Rosser et al. 2021).

Patterns of genetic variation were partly consistent with classical island biogeography predictions, but were also partly counterintuitive. Nucleotide diversity, which measures intrapopulation genetic variation, was lower on islands than in CAEs, consistent with the paradigm that genetic diversity scales with ongoing gene-flow, affecting both demographic and evolutionary processes. Larger and core areas sustain higher genetic diversity than smaller islands or peripheral regions (Wang et al. 2014; De Kort et al. 2021), especially in recently established populations (James et al. 2016). Larger populations also buffer stochastic demographic fluctuations, thus reducing extinction probability, in turn facilitating long-term divergence (Lomolino 2000; McCulloch and Waters 2019). Although populations on CAEs had significantly higher *NucDiv* than on islands, we found no consistent increase in haplotype diversity with island area, despite a four-order-of-magnitude range in sizes (from Gorgona at 2.2 km^2 to Sicily at 25,711 km^2).

This suggests that most genetic diversity likely originates on the mainland, where new variants can spread widely, sometimes mixing with or replacing older lineages through demographic expansion, lineage turnover or processes involving endosymbionts such as *Wolbachia* (e.g., Arif et al. 2021 for Palearctic butterflies), but is dramatically halted at sea straits. Change in lineage distribution over mainland in our study region were reconstructed over millennial timescales (*Maniola jurtina*; Dapporto and Bruschini 2012), or even within the past century (*Hyles euphorbiae* complex; Mende and Hundsdoerfer 2013). This hypothesis is also supported by the observation that endemic mutations show similar frequencies on islands and in CAEs. In fact, the circum-Mediterranean region shows comparable high level of local genetic endemism across islands and mainland, due to glacial refugia and the 'refugia within refugia' phenomenon (Gómez and Lunt 2007). Notably, the hotspots of genetic endemicity in Mediterranean mainland regions are similar in size to the largest islands (Dapporto et al. 2024). In addition, many endemic haplotypes in non-fully endemic populations may simply reflect the star-like pattern of mitochondrial DNA. In this sense, endemic mutations may partly correlate with nucleotide diversity, which is higher in CAEs.

Although islands do not show a particularly high proportion of endemic mutations, populations separated by sea straits were significantly more differentiated (*Dst*) than those separated by land. Strong genetic contrasts also occur across straits that were land bridges during the Last Glacial Maximum (e.g., Sardinia-Corsica and Sicily-Italian mainland). The relatively short isolation during the current interglacial (~11,000 years) supports the hypothesis that many current vicariant patterns were established recently (Dapporto et al. 2012; Mende and Hundsdoerfer 2013; Scalercio et al. 2020; Arif et al. 2021). Island populations may retain ancestral lineages for a longer time than CAEs. For example, Corsica, the circum-Italian islands and the Italian Peninsula tend to host butterfly lineages typical of mainland Europe, whereas Sardinia and Sicily (the two largest islands) share many potentially ancestral lineages with North Africa and/or Iberia (Vodă et al. 2015; Livraghi et al. 2018; Scalercio et al. 2020). This likely reflects persistence of ancestral island populations, while mainland ones were replaced after the LGM by new colonisers (Hinojosa et al. 2022) or by lineage turnover (Dapporto and Bruschini 2012; Arif et al. 2021).

Genetic sweeps, where competitive lineages replace existing ones before reproductive isolation occurs, can reduce regional genetic diversity, especially in mtDNA due to hitchhiking (Gillespie 2001). On the mainland, such sweeps may occur rapidly, eroding differentiation between CAEs and nearby areas over distances < 200 km.

Large islands, even when separated by narrow sea straits (e.g., Sardinia-Corsica or Sicily-peninsular Italy), may resist genetic homogenisation and replacement longer than smaller but more isolated islands (Scalercio et al. 2020), as fixation is a slow process when immigrants must integrate in large stable populations and stochastic losses outweigh fixation. In contrast, small islands are more susceptible to extinction of ancestral populations, recolonisation from the nearest mainland or replacement by immigrants, leading to greater genetic similarity with nearby source populations (Lomolino 2000).

Alongside island features, some species' functional traits (e.g., phenology, migratory behaviour and wing size) contributed to explaining various aspects of genetic variation. Since the adult stage is the dispersive phase in butterflies, it is expected that large, migratory species and/or with longer flight periods are less likely to maintain geographically restricted haplotypes, both on islands and on the mainland (Scalercio et al. 2020; Dapporto et al. 2024; Sucháčková Bartoňová et al. 2024). In most cases, species traits significantly explain genetic variation through interactions with geographic variables and region type. Overall, the effects of functional traits aligned with expectations (Burney and Brumfield 2009; Papadopoulou et al. 2009; Gálvez-Reyes et al. 2021; Dapporto et al. 2024), with reduced genetic structure in species with higher dispersal capacity, but only on islands. In contrast, trait effects were weaker in CAEs.

Migratory behaviour was a strong predictor of genetic differentiation (*Dst*): migratory species consistently exhibited low spatial genetic structure, while sedentary species showed increasing divergence with island area. Although migrants can harbour high levels of intraspecific genetic diversity, this is typically structured in star-like mtDNA networks that support moderate nucleotide diversity (García-Berro et al. 2023) and a few endemic mutations, but not persistent spatial differentiation. Recurrent long-distance dispersal homogenises genetic variation across regions. Similarly, wing size, a proxy for dispersal ability, also mattered: smaller butterflies had higher EM in larger areas, confirming previous findings (Dapporto et al. 2024). However, this pattern was absent in CAEs, again suggesting that sea barriers interact with species traits more strongly than terrestrial distances to shape genetic diversity. Phenology explained a similar increase in EM with area in species with a short flight period between islands and CAEs, a pattern disappearing in CAEs when species with a long flight period are considered.

4.2 | The Relationship Between Community Composition, Species Traits and Genetic Variation

Our findings for Objective 2 indicate that the observed increase in genetic differentiation with island area and isolation is driven by a combination of community-level and population-level processes, with a stronger signal associated with the former. This comparison does not represent a formal partitioning of variance, but rather an assessment of the relative strength of two non-exclusive processes linking island features to genetic variation. Thus, the higher R^2 values of the community-level models should be interpreted as evidence of a stronger compositional signal, rather than as a measure of its exclusive contribution. Specifically, the separation of community and population effects with isolation showed counterintuitive results which can explain the absence of a relationship between increasing strait length and genetic variation. In fact, in our nested systems, richer islands (the larger and less isolated ones) also supported rarer species with higher genetic divergence. It has to be noted that the Western Mediterranean islands do not represent the most extreme cases of colonisation filters for European butterflies. For instance, the highly isolated Azores archipelago in the Atlantic (not included in this study) hosts six migrant butterfly species: *Danaus plexippus*, *Colias crocea*, *Pieris brassicae*, *Lampides boeticus*, *Vanessa atalanta* and *Vanessa cardui* with no evidence of differentiation on this archipelago. The only non-migratory species complex is the endemic, highly differentiated, *Hipparchia azorina*. Within our dataset, two informative examples are Montecristo and Ustica. Despite their small size and isolation, they support 12 and 15 butterfly species, respectively. In both cases, only about half of them (seven on Montecristo and eight on Ustica) are migrants, illustrating how community assembly in the Western Mediterranean can include a balanced number of migratory and non-migratory taxa even under the filters of relatively strong isolation and small areas. Despite such balanced community composition, increasing area and isolation have contrasting effects on species richness and in shaping the genetic variation of species composing a community. The positive relationship between area and genetic divergence was expected (see Objective 1), while the negative relationships between genetic divergence and isolation at community and mostly at population level (populations of the same species are less diverging in distant compared to less isolated islands) are counterintuitive in the perspective of island biogeography paradigms. However, these findings reinforce the hypothesis that isolated (and small) Mediterranean island populations are subjected to frequent extinction re-colonisation events. In this perspective, persistence can be favoured by events of dispersal in less isolated islands. Gene flow can enhance genetic variation by: (i) providing raw material for natural selection and (ii) increasing population persistence due to rescue effect and reduced inbreeding, thus allowing long-term divergence (Warren et al. 2015). In mammals, even low mainland immigration may maintain genetically distinct island populations (Steinbach et al. 2018). Moreover, it must be noted that mitochondrial DNA reflects only matrilineal history, and male-biased dispersal may increase gene flow and reduce island-mainland genetic contrasts without reducing mtDNA diversity. Mark–recapture studies provide mixed evidence regarding male-biased dispersal in butterflies, but strong flight effort, even below what is needed to cross a sea barrier, can lower female fecundity (Gibbs et al. 2010), potentially reducing female-mediated gene flow to negligible levels.

5 | Conclusion

By applying the concept of continental area equivalents (CAEs), we show that butterfly populations exhibit contrasting genetic patterns across even narrow sea barriers relative to comparable distances on the mainland. Several patterns were consistent with classical biogeographic expectations, including increased genetic differentiation (*Dst*) in the presence of a sea barrier and with increasing island area, as well as higher nucleotide diversity on the mainland. However, we found no evidence for increased genetic endemism on islands compared to CAEs. Instead, only large and relatively less isolated islands, such as Sicily, host sufficient endemic lineages to generate substantial genetic differentiation.

These results indicate that, in a system dominated by fragment islands within a semi-enclosed sea, genetic differentiation is driven primarily by processes occurring on the mainland—namely the generation and spread of genetic variation—rather than by in situ diversification on islands. The strong effects of island area and dispersal-related traits likely reflect differences in extinction and recolonisation dynamics, which are more frequent on small islands and reduced on larger ones. Although mitochondrial DNA captures only maternal lineages (Galtier et al. 2009), previous studies across Western Mediterranean butterflies have shown largely congruent patterns between mitochondrial and nuclear markers (Vodă et al. 2015; Livraghi et al. 2018). While genomic data will ultimately provide a more complete picture, mtDNA remains a powerful tool for detecting broad-scale patterns of genetic differentiation and for guiding future genomic investigations. Overall, our findings suggest that island genetic patterns emerge from the interplay between mainland-driven processes and insular filtering, with islands acting less as centres of diversification and more as selective environments that retain or exclude lineages shaped elsewhere.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The COI sequences are available in BOLD, Genbank and in the iodatabase repository https://github.com/leondap/iodatabase. The newly generated sequences are also available in the BOLD project DS-MEDIS https://dx.doi.org/10.5883/D-SMEDIS. The script and data to replicate the analyses are available at Zenodo https://doi.org/10.5281/zenodo.19487359.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1.1.** The main R libraries and functions used for calculating genetic variation indexes and their sources. **Table S1.2.** The Pearson correlations among the explanatory variables computed among species traits (grey) and island features (white). **Table S1.3.** The result of the decomposition of community-level (compostional) and population-level (within species) processeses for Area and Strait Length (Dist) for the three considered genetic indexes. Result2 for the total effect of the two processes (Total effect) and the relationship between *Dst*, EM and *NucDiv* with species incidence are also reported. **Figure S1.1.** Heatmap of *Dst* values across species (columns) and islands (rows), with species ordered from widespread to rare and islands ordered by increasing area. Colours represent *Dst* values, with warmer colours indicating higher divergence. Grey crossed cells indicate species–island combinations for which *Dst* could not be estimated. **Figure S1.2.** Heatmap of *Dst* values across species (columns) and islands (rows), with species ordered from widespread to rare and islands ordered by decreasing minimum distance from the nearest source. Colours represent *Dst* values, with warmer colours indicating higher divergence. Grey crossed cells indicate species–island combinations for which *Dst* could not be estimated. **Figure S1.3.** Heatmap of EM values across species (columns) and islands (rows), with species ordered from widespread to rare and islands ordered by increasing area. Colours represent EM values, with warmer colours indicating higher endemicity. Grey crossed cells indicate species–island combinations for which EM could not be estimated. **Figure S1.4.** Heatmap of EM values across species (columns) and islands (rows), with species ordered from widespread to rare and islands ordered by decreasing minimum distance from the nearest source. Colours represent EM values, with warmer colours indicating higher endemicity. Grey crossed cells indicate species–island combinations for which EM could not be estimated. **Figure S1.5.** Heatmap of *NucDiv* values across species (columns) and islands (rows), with species ordered from widespread to rare and islands ordered by increasing area. Colours represent *NucDiv* values, with warmer colours indicating higher endemicity. Grey crossed cells indicate species–island combinations for which *NucDiv* could not be estimated. **Figure S1.6.** Heatmap of *NucDiv* values across species (columns) and islands (rows), with species ordered from widespread to rare and islands ordered by decreasing minimum distance from the nearest source. Colours represent *NucDiv* values, with warmer colours indicating higher endemicity. Grey crossed cells indicate species–island combinations for which *NucDiv* could not be estimated.