1 **Rise and fall of island butterfly diversity. Understanding population diversification and**

2 **extinction in a highly diverse Archipelago.**

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43 **Short running title:** An integrated dataset to study island biodiversity

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ABSTRACT

Aim

We described fine-scale diversity patterns of the entire butterfly fauna occurring on the

Tuscan Archipelago. By assessing the traits associated with population diversification,

haplotype uniqueness and extinction, we aimed to identify the factors determining the origin

and the maintenance of genetic diversity and population vulnerability to environmental

changes.

Location

Tuscan Archipelago, Sardinia, Tuscany (Italy) and Corsica (France).

Methods

We built a mtDNA dataset (1303 COI sequences) for the 52 butterfly species reported in the

Archipelago also including specimens from neighbouring areas and compiled data on 12

species traits and on the apparent extinction of butterfly species from the main islands. We

calculated different indices indexes of genetic differentiation, and using stepwise

phylogenetic regressions we evaluated the relationships between these indices and species

traits. Finally, we inferred which traits are associated with apparent extinction of species on

individual islands using phylogenetic regression.

Results

The overall spatial pattern of genetic diversity corresponded with the proximity of the areas,

but strong contrasts were also identified between geographically close areas. Together with

the island endemics, several common and widespread species revealed a high genetic

71 diversification among islands and mainland. Phylogenetic regressions showed that smaller-

72 sized, more specialized species, with a preference for drier regions, displayed greater genetic

73 structure and/or haplotype uniqueness. Capraia has particularly suffered loss of diversity,

74 which significantly affected species with shorter flight periods.

75 **Main conclusions**

76 Tuscan island butterflies are characterized by strong genetic contrasts and species differ in

77 their contribution to the overall genetic diversity. By ranking the species for their contribution

78 to genetic diversity and by identifying the traits linked to the emergence and maintenance of

79 diversity, we provided a valuable tool to prioritize the populations as targets for monitoring

80 and conservation actions. The large dataset we provided represents a resource to test

81 biogeographical hypotheses.

INTRODUCTION

The worldwide biodiversity crisis calls for the identification, prioritization and protection of biodiversity hotspots, and understanding how biodiversity is generated and lost determines the success of this endeavour (Dirzo et al., 2014; Venter et al., 2014). The wealth of information now available in the "big data era" greatly facilitates these efforts, especially the ability to work at an unprecedented resolution (Hampton et al., 2013). For conservation biogeography this includes the increased availability of molecular, occurrence and trait data for various organisms (Ladle & Whittaker, 2011; Fernández-Palacios et al., 2015).

Islands host a disproportionate fraction of global biodiversity, often characterized by distinctive faunas (Whittaker & Fernández-Palacios, 2007), and therefore represent a model system to better understand general patterns in biogeography. Insular populations are typically exposed to high risks of extinction since restricted habitats can easily become unsuitable because of stochastic events or human disturbances acting at both local (habitat fragmentation, alteration, invasion by alien species) and global scales (e.g. climate change) (Fordman et al., 2010). Understanding the factors that drive the emergence, maintenance and loss of island biodiversity is crucial for planning and implementing evidence-based conservation prioritization and protection measures (Ladle & Whittaker, 2011).

Remote oceanic islands experience infrequent colonization events followed by genetic drift and adaptive radiation (Rosindell & Phillimore, 2011). In contrast, biotas on less isolated islands are mostly assembled following frequent events of colonization and extinction, which produce nested communities of the source species with few endemics (Whittaker & Fernández-Palacios, 2007). However, a pace of extinction and colonization on islands (turnover) slower than the dynamics occurring in the surrounding regions can generate intraspecific diversification and relictuality, which creates community distinctiveness among

106   islands and from neighbouring mainlands (Masini et al., 2008; Dapporto et al., 2012). Such

107   diversity is usually represented by cryptic species or genetic lineages that can be detected only

108   after in-depth molecular and/or morphologic analyses (Hernández-Roldán et al., 2016; Vodă

109   et al., 2015a, 2016).

110   The occurrence of endemic and relict populations can be the result of deterministic processes,

111   largely affected by species characteristics. According to a widely accepted paradigm of island

112   conservation biogeography, genetic diversification and extinction probability of island

113   populations are inversely related to their degree of mobility and generalism (Burney &

114   Brumfield, 2009; Dennis et al., 2011; Salisbury et al., 2012; Dawson et al., 2014 but see also

115   Kobayashi & Sota, 2016 for different patterns). This hypothesis has profound implications for

116   conservation ecology because populations with unique genetic fingerprints are clearly

117   irreplaceable but potentially suffer from high extinction probability (Ricklefs, 2009).

118   Nevertheless, this has been rarely tested (Burney & Brumfield, 2009; Vodă et al., 2016),

119   probably because of the absence of integrative datasets combining molecular and occurrence

120   data with species traits for entire species-rich taxonomic groups and from a large geographic

121   area.

122   Here we tested if species having low dispersal capacities and restricted ecological

123   requirements tend to colonize islands at a slower rate, experience reduced gene flow and thus

124   have i) higher diversification rates and ii) higher likelihood of local extinction compared with

125   more mobile and generalist species. We designed an integrated framework (Fig. 1) based on a

126   dataset comprising: i) a revised distribution list of the 52 butterfly species reported for the

127   Tuscan Archipelago and the apparent disappearance of several species in the last 115 years, ii)

128   1303 cytochrome c oxidase subunit I (COI) sequences (658 bp) for all the species, including

129   populations from the surrounding areas (Sardinia, Corsica and Tuscany), and iii) 12 species

130 traits related to mobility, phenology, trophic generalism and climatic preferences. The

131 analysis of this dataset allowed us to: i) describe the patterns of population diversification

132 (genetic diversity and haplotype uniqueness) among islands and neighbouring areas, ii)

133 identify the functional traits that are correlated with the emergence of diversification and

134 species disappearance on islands. Finally, iii) we ranked the species according to their

135 contribution to the overall island diversity. Our integrated framework (Fig. 1) allowed us to

136 provide evidence-based guidelines for butterfly conservation in a well-known Mediterranean

137 diversity hotspot (Dennis et al., 2008).

138

139 METHODS

140 *Study area and occurrence data*

141 We carried out annual surveys of the butterflies of the Tuscan islands and the neighbouring

142 areas of Sardinia, Corsica and Tuscany between 2000 and 2015 (Fig. 1a). We sampled the

143 main biotopes from early spring to late autumn, with different tools (mostly Malaise traps and

144 insect nets).We compared occurrence data from our surveys with published records dating

145 back to 1900 (Appendix S1). Sampling was not done constantly throughout the 15 years but

146 our extensive collections represent the most intensive sampling effort done on the butterflies

147 of these islands, therefore it is reasonable to assume that a species reported in the past but not

148 during the last decade has either become extinct or has significantly declined. We restricted

149 our analysis of species disappearance to the three largest islands: Elba, Capraia and Giglio, for

150 which sufficient historical data were available.

151

Genetic assessment

Using standard sequencing procedures (deWaard et al., 2008), we obtained 1303 COI sequences for specimens belonging to the 52 butterfly species reported for the Tuscan islands between 1900 and 2015, including specimens from Sardinia, Corsica, Argentario and four mainland areas (Fig. 1a). We also used 2940 sequences from other parts of Europe and Asia. Information about specimens and sample size for each population is available in the "dataset.csv" file (Appendix S2). All sequences were aligned in Geneious 6.0.6 (www.geneious.com).

The butterfly species currently recognized by taxonomists, and for which trait data are available, show varying levels of intraspecific genetic divergence, including potential cases of cryptic taxa (Dincă et al., 2015). We considered as separate units most species recognized by the widely accepted checklist of the Fauna Europaea Project (Karsholt & Van Nieukerken, 2013; www.faunaeur.org) and also considered as single units taxa displaying a minimum COI p-distance lower than 3%. In butterflies, this threshold separates more than 90% of the recognized species (Hebert et al., 2003) and a recent study on Sicilian islands confirmed this observation (Vodă et al., 2016). We also repeated all the analyses without setting any distance threshold, and considered as single units only the species recognized by Fauna Europaea.

 We calculated the genetic uncorrected p-distances among all sequenced specimens for each species by using the function "dist.dna" of the "ape" R package. We preferred p-distance to tree-based genetic distances because, at the intraspecific level, coalescence has not taken place and distances calculated on branch lengths of bifurcating phylogenetic trees do not properly reflect the reticulated evolutionary processes (e.g. Posada et al., 2001). Moreover, recent reviews indicate that p-distances are the best option in the analysis of COI data compared to other indices (Srivathsan and Meier, 2012). Based on p-distances, we obtained

176   two measures for population differentiation: Dst and Gst (Nei, 1987). We also calculated the

177   pairwise Gst among all pairs of populations for each species (see Appendix S1 for a

178   description of the indices).

179   Based on the Gst pairwise matrices we produced for each species the mean Gst matrix,

180   representing the degree of differentiation among areas based on all species. A Principal

181   Coordinates Analysis (PCoA) was applied to this matrix to obtain the overall diversity pattern

182   among areas. Subsequently, we aligned this configuration with the geographic location of the

183   areas by using the "procrustes" analysis from the "vegan" R package and tested the correlation

184   between the PCoA configurations of Gst and the spatial location by using the vegan function

185   "protest". To visualize the pattern of similarity among islands in the geographic space, we

186   projected the PCoA configuration in RGB space using the R package "recluster" (Dapporto

187   et al., 2014). The colour resemblance of the resulting configuration is directly proportional to

188   the genetic similarity among the communities.

189   Four species (*Leptidea sinapis*, *Aglais urticae*, *Nymphalis polychloros*, *Argynnis paphia*)

190   recorded for Tuscan islands in the last century but not during our surveys have also been

191   analysed by computing the Dst and Gst between Tuscany, Sardinia and Corsica to estimate

192   the fraction of diversity that has presumably disappeared.

193   Island haplotype uniqueness for each species was calculated as:

194    $$Uni = \sum_{h=1}^{n} \frac{\min(D_{h,m})}{occ}$$

195   where h are the haplotypes found on the Tuscan islands and not recorded on mainland areas of

196   Europe and Asia, $\min(D_{h,m})$ is the p-distance between the hth haplotype and the genetically

197   closest haplotype from mainland, and occ is the number of islands (Tuscan islands, Sardinia

198   and Corsica) where the hth haplotype has been found. Uniqueness for a given species is higher

199   when: 1) there are many insular endemic haplotypes, and/or 2) they show high divergence

200   with respect to the closest mainland haplotype and/or 3) they are found in fewer islands.

201   Uniqueness values (Uni) were calculated for each species occurring on any island except for

202   the island endemics that do not have mainland populations (*Hipparchia aristaeus* and *H.*

203   *neomiris*).

204   To assess the importance of each species for the diversity of the Tuscan Archipelago we

205   ranked species according to Gst, Dst and Uni and subsequently summed the ranks. The

206   patterns of genetic variation were also analysed by inferring maximum parsimony haplotype

207   networks using the program TCS 1.21, with a 95% connection limit for all species except *H.*

208   *neomiris* (94% connection limit) and *Coenonympha corinna* for which we used a fixed

209   connection limit of 23 steps (Clement et al., 2000).

210

211   *Species traits and phylogenetic regressions*

212   Review studies suggest that only morphological, physiological or phenological features that

213   can be measured on individual organisms and without reference to the environment or any

214   other level of organization should be considered as functional traits (Violle et al., 2007;

215   Moretti et al., 2016). Moretti et al. (2016) identified a series of 29 functional traits to cover

216   the primary functions of invertebrates, divided into five major groups: morphology, feeding,

217   life history, physiology and behaviour. Measuring the 29 traits on individuals for all the

218   studied species would have entailed a major long-term effort that was beyond the scope of

219   this study. Thus, based on literature data and personal observations, we assessed 12 species

220   traits representing four of the five groups (excluding behaviour) identified by Moretti et al.

221   (2016): but encompassing morphology, feeding, life history and physiology. For each trait we

222   formulated functional hypotheses (Table 1): a) Trophic generalism (feeding), was identified

223   as i) the number of host plant genera reported in the literature; b) Mobility was assessed with

224   a morphological traits represented by ii) wingspan and obtained as the average between

225   minimum and maximum size reported in the literature; c) Phenology (life history traits) was

226   identified as iii) the length of the flight period, iv) the first month when adults emerge, v) the

227   last month when adults fly, and vi) voltinism. Finally, d) climatic preference and tolerance

228   (physiology) were assessed by proxy variables for eco-physiological responses to

229   environmental conditions. These variables have been calculated by Schweiger et al. (2014) by

230   modelling species distribution in Europe based on occurrence data, and then by averaging

231   temperature and precipitation among the spatial cells where each species is predicted to occur.

232   Although these indices cannot be considered as strict functional traits since they are obtained

233   from the geographic distribution of the species (Violle et al., 2007), they are widely

234   recognized as proxies for the traits responsible for eco-physiological responses to climate (e.g.

235   Devictor et al., 2012). The variables we included are: vii) mean annual temperature viii) and

236   precipitation, ix) standard deviations of the temperature mean and x) and precipitation, xi)

237   upper 95% confidence limit of temperature mean, and xii) lower 95% confidence limit of

238   precipitation mean.

239   Butterfly traits are usually highly inter-correlated but they can be conveniently reduced to

240   factors by using ordination methods (Carnicer et al., 2013; Dapporto & Dennis, 2013). For

241   morphology, life history and physiology traits we applied a Principal Component Analysis

242   (PCA), using the R function "rda" and the components with eigenvalues higher than one have

243   been used as variables for successive analyses. Some literature sources did not report the

244   wingspan for all the studied species and we imputed the missing values by using the "mice"

245   function of the "mice" R package (see Appendix S2). The algorithm imputes an incomplete

246   variable by generating plausible values based on other variables in the data by Multivariate

247   Imputations by Chained Equations (MICE) (Van Buuren & Groothuis-Oudshoorn, 2011).

248   The existence of a phylogenetic signal for the variables of each trait following the PCA

249   ordination and for Dst, Gst and Uni was tested with Pagel's lambda index by applying the

250   "phylosig" R function of the "phytools" package. Gst, Dst and uniqueness have been mapped

251   onto the phylogenetic tree by using the "contMap" function of the "phytools" package.

252   Character mapping is accomplished by estimating states at internal nodes using maximum

253   likelihood and then by interpolating the states along each edge (Ravell, 2013). The

254   relationships between the variables and Dst, Gst and Uni have been assessed using

255   phylogenetic stepwise regressions. We also employed Pagel's lambda as a model for the

256   phylogenetic covariance of residuals and applied a two-way selection of variables based on

257   the Akaike Information Criterion (AIC) as implemented in the function "phylostep" of the

258   package "phylolm". From the phylogenetic regressions we removed the species not recorded

259   for the Tuscan Archipelago during the study period since no DNA sequences were available.

260   We square-root transformed Dst and Uni to improve their normality and standardized the

261   values of the traits with zeta-scores to provide a balanced contribution to the phylogenetic

262   regression.

263   The importance of traits in explaining possible extinctions of butterflies on two islands (Elba

264   and Capraia) has been assessed with a logistic phylogenetic Generalized Linear Model using

265   the function "phyloglm" of the package "phylolm", in which species found during our surveys

266   and species that have not been confirmed, represented the binary response variable, and the

267   trait variables the predictors. Logistic GLM was not performed for Giglio as only three

268   species disappeared on this island. "Phylolm" function was used to assess if species

269   disappeared in at least one island showed higher values of Gst, Dst and Uni

270   .

271 As a reference phylogeny, we used the Maximum Likelihood (ML) phylogenetic tree based

272 on COI sequences for all the western Mediterranean butterflies, freely available in the

273 package "recluster" (Dapporto et al., 2013). The tree was inferred with topological constraints

274 at family and subfamily levels following the butterfly phylogeny (see Appendix S1 for

275 details). ML analyses were performed using RAxML BlackBox (Stamatakis et al. 2008). A

276 GTR+Gamma+I model was selected and node supports were assessed through 100 rapid

277 bootstrap replicates. Effect size for models has been evaluated by plots of observed vs fitted

278 values associated with Spearman rho correlation.

279

280 RESULTS

281 Based on the taxonomy proposed by Fauna Europaea and by applying a 3% threshold of COI

282 divergence, we identified 52 units among the taxa reported in literature for the Tuscan Islands

283 (hereafter 'species', Table 2). During our surveys on these islands we recorded a total of 46

284 species. The comparison between observations during the last decade and literature data from

285 1900 to 2000 for Elba, Giglio and Capraia, identifies those species (see Table 2) that

286 probably became extinct or strongly declined on these islands (respectively six, three and

287 seven on Elba, Giglio and Capraia)..

288 Dst was correlated with both Gst (Spearman rank test: rho 0.836, P<0.001) and Uni (rho

289 0.460, P<0.001), while Gst was not correlated to Uni (rho 0.192, P=0.213). Gst values showed

290 an almost bimodal distribution (14 species with Gst<0.25 and 10 species with Gst>0.75, Table

291 2). Twenty-four species had haplotypes not recorded on the mainland and most of these

292 species did not belong to endemic taxa (Table 2). Gst and Uni did not have a significant

293 phylogenetic signal (lambda 0.218; P=0.314 and lambda <0.001; P=1.000, Fig. 2), while Dst

294   had a significant effect (lambda 0.334; P=0.034; Fig. 2). Ordering species by the sum of ranks

295   of the three indices (Dst, Gst and Uni) showed that, together with endemics, several common

296   and widespread species provided a large contribution to diversity. The first quartile of the top

297   ranking species comprised four species/groups with endemic elements in the Tuscan islands

298   (*C. corinna*, *Lasiommata megera/paramegaera*, *H. neomiris*, *Aglais urticae/ichnusa*) and ten

299   widespread species, most of them ubiquitous in Europe and not included in any protection list

300   (Table 1).

301   The wingspan measures reported in the four literature sources used were highly correlated

302   (Pearson R>0.9 for all pairs) and the PCA identified only one component with an eigenvalue

303   higher than one (Table 1, Figure S46). For life history and physiologic traits two components

304   were considered (Table 1, Figure S47). The first phenological component was mainly linked

305   to the length of the flight period (voltinism, number of months when adults occur), while the

306   second was mainly linked to seasonality (first and last month of emergence). The first

307   component for physiologic traits ordered species from those experiencing high temperatures

308   and low precipitation to those living in colder and wetter areas, while the second component

309   ordered species mostly according to their precipitation tolerance (Table 1, Figure S48). The

310   six resulting variables showed a lower correlation among each other with Pearson correlation

311   values always lower than 0.400 (Table S1). Among the six resulting variables, the number of

312   host plants, phenology PC1, physiology PC1 and PC2 did not show a phylogenetic signal

313   (lambda 0.282; P=0.228; lambda<0.001; P=1.000; lambda 0.062; P=0.711; lambda<0.001;

314   P=1.000, respectively), while wingspan PC1 and phenology PC2 showed a significant effect

315   (lambda 1.187; P<0.001; lambda 1.083; P<0.001, respectively).

316   The overall spatial pattern of genetic variation based on Gst corresponded to the proximity of

317   the areas but with a rather low level of correlation (protest correlation 0.592, P=0.015, Fig.

318   3a,b).

319   The AIC procedure for the stepwise phylogenetic regression for Gst selected a model with

320   four variables, but only mobility and trophic generalism had a significant effect (Table 3),

321   meaning that smaller-sized and more generalist species had a higher Gst. For Dst, four

322   variables entered the model - three were significant and showed that smaller-sized, more

323   generalist species and those experiencing less annual precipitation had a higher variation

324   (Table 3). Two variables entered the Uni model showing that species living in drier areas

325   significantly had higher haplotype uniqueness (Table 3), while trophic generalism entered the

326   model but without significant effect. Plots for observed vs fitted values of the three models

327   (Fig. S53) showed large residuals indicating that Gst, Dst and Uni are only weakly explained

328   by the measured traits (Spearman rho: Gst 0.537, Dst 0.430. Uni 0.368).

329   Species that had disappeared from at least one island showed significantly higher values of

330   Dst, while no differences in Gst and Uni were found (Table 3).

331   According to the logistic phylogenetic GLM, species that disappeared from Capraia had lower

332   values in PC1 for phenology, corresponding to shorter flight periods (Table 4). A plot for

333   observed vs fitted values (Fig. S54) revealed a good fit for this analysis (Spearman rho 0.798).

334   For Elba we found no significant effect explaining the disappearance of the six species (Table

335   4).

336   The analyses in which we used only the taxonomy from Fauna Europaea returned very similar

337   results to the ones in which we used a 3% threshold for species identification (see Table S2-

338   S5 and Figure S55 in Appendix S1).

339

DISCUSSION

The integration of an updated taxonomic list, species occurrence spanning across 115 years (1900-2015), mitochondrial DNA sequences and species traits, allowed us to characterize the butterfly diversity in the Tuscan Archipelago at an unprecedented resolution and to infer which species traits explain the rise and decline of butterfly diversity in this archipelago.

An area of biogeographic contrasts

The Tuscan Archipelago is an insular hotspot for butterfly diversity and stands out among European islands for hosting far more endemics than would be expected based on their geography (e.g. area and isolation) (Dennis et al., 2008), resulting in the highest priority for butterfly conservation among circum-Italian islands (Dapporto & Dennis, 2008). Its unexpected level of endemicity and richness is a consequence of its intermediate location between Tuscany and the Sardo-Corsican region, generating a double filtering effect (Dapporto & Cini, 2007; Fattorini, 2009).

We found that a main determinant for the genetic make-up of populations is island location (see the protest analysis), the same as for the community composition at species level (Dapporto & Cini, 2007). Nevertheless, a model based only on a double filtering effect is not sufficient to explain the observed degree of genetic diversity. In fact, we identified strong contrasts between geographically close areas, such as between Montecristo (very similar to the Sardo-Corsican region) and Pianosa (more similar to Elba and the Italian Peninsula), which are separated by 30 km; between Capraia and Elba (same pattern as before, distance 33

361 km) and between Giglio and Argentario, separated by only 14 km (Fig. 3). Striking

362 divergence among populations from nearby areas is unexpected in butterflies characterized by

363 a high mobility resulting in a high capacity to track suitable environments (Wilson et al.,

364 2010; Waters, 2011; Devictor et al., 2012). However, in the western Mediterranean (Tuscan

365 islands included), chequered distributions of sister species and genetic lineages are a common

366 phenomenon, probably due to the combination of several historical determinants and

367 contemporary ecological forces (e.g. the connection between land masses during the Last

368 Glacial Maximum, density-dependent phenomena, differences in climatic and environmental

369 features, Vodă et al., 2015a,b, 2016). Accordingly, more than 30% of the examined species,

370 comprising both endemic and widespread taxa had a Gst value higher than 0.5.

371 Endemic taxa also have unexpected patterns of intraspecific genetic diversity among islands.

372 *Coenonympha corinna/elbana* is highly divergent among the three clades they form in: i)

373 Tuscany, Elba and Giannutri; ii) Capraia and Corsica; and iii) Sardinia. This pattern only

374 partially supports the current taxonomic separation into two species or subspecies (*C.*

375 *corinna*: Sardinia, Corsica and Capraia; *C. elbana*: Elba, Tuscany and Giannutri) (Fig. 3c).

376 *Hipparchia neomiris*, an endemic species from Sardinia, Corsica and Elba, also displayed

377 notable intraspecific divergence. COI sequences from Elba and Corsica are differentiated by

378 at least 2% compared to conspecific individuals from Sardinia. By contrast, no genetic

379 diversification was detected in the *Plebejus idas* group, since the endemic *P. bellieri* from

380 Sardinia and Corsica (recognized as a good species in Fauna Europea) shared COI barcodes

381 with the population from Elba, treated as a species in some works, and with the mainland

382 populations, elsewhere reported as as *P. idas* or *P. abetonicus* (Balletto et al. 2015).

383 Phylogenetic regressions suggest that the species adapted to a dry climate, the small-sized

384 ones and the host plant specialists showed a higher degree of island uniqueness and of genetic

385   diversification among islands and populations. According to our hypotheses, these species

386   traits can facilitate the emergence and maintenance of these genetic contrasts since typical

387   Mediterranean species thrive on islands, while species with reduced dispersal and poor

388   colonization capabilities probably experience reduced gene flow.

389   Several species previously recorded on three Tuscan islands were not observed in the last 10

390   years, suggesting that they became extinct or declined considerably (Table 2). On Giglio only

391   three species have not been recorded during the last decade, but they include *C. corinna,* the

392   most emblematic species for the archipelago, as well as *A. agestis* and *Polyommatus icarus,*

393   both showing high levels of population diversification (Table 2).

394   Elba has apparently lost six species. In this case as well, some of the taxa have diversified

395   populations in the study area (*Aglais urticae/ichnusa, Leptidea sinapis* and *Nymphalis*

396   *polychloros* showed a Gst higher than 0.4). For this island we found no species traits

397   correlated with disappearance and the fraction of likely extinct species (11.5%) was lower

398   than on Capraia 30% of the species reported in the past have disappeared.

399   Two species disappeared from Capraia were insular endemics (*H. neomiris* and *H. aristaeus*)

400   and others showed divergent populations in the study area (*P. cecilia*, *L. phlaeas*, *M. jurtina*).

401   Disappeared species represented a fraction of the fauna with a short flight period. As the

402   length of the flight period is correlated to inter-island dispersal in the Tuscan Archipelago

403   (Dapporto et al., 2012).,species with a short flight period have a lower probability of re-

404   colonizing from surrounding areas. Interestingly, there are no typical springtime species on

405   Capraia and taxa with short flight periods are typically monovoltine, with adults emerging at

406   the beginning of summer, aestivating during the hottest weeks and laying eggs in

407   September/October. Aestivation in the Mediterranean region is known for *M. jurtina* (Scali,

408 1971) and *Hipparchia semele* (García-Barros, 1988), a species closely related to *H. aristaeus*.

409 Both of them have disappeared from Capraia, together with two other Satyrinae (*P. cecilia*

410 and *H. neomiris*), which tend to be frequent in woodlands and scrub/maquis during the hottest

411 and driest months. Because there are barely any remaining woods on Capraia, aestivation may

412 represent an important stress period with current temperature increases (Shreeve et al., 2009;

413 Cerrato et al., 2016). Compared to Elba and Giglio, Capraia is more isolated and this could

414 have hampered a rescue effect for many species. Moreover, Elba has the highest mountain

415 peak among the small Italian islands (Monte Capanne, 1019m) and woodlands are common

416 on both Giglio and Elba. The higher environmental heterogeneity of Elba and Giglio could

417 have provided a wider range of suitable areas for many species under environmental stress

418 and climatic oscillations.

419 Extinction events over long periods are expected on islands based on the equilibrium theory

420 (MacArthur & Wilson, 1967), but they should be paralleled by colonization events, which

421 was not the case for the Tuscan islands. On Elba only *Anthocharis cardamines* has been

422 discovered after 1950; on Capraia only *P. aegeria* and *A. agestis* have been found after

423 intensive field research between 1970-1980, when almost all the seven extinct species were

424 present; only *C. rubi* has been recently discovered on Giglio.

425 *Guidelines for the conservation of the Tuscan Archipelago butterflies*

426 Ranking species according to their contribution to genetic diversity (Table 2) shows that

427 butterfly diversity in the Tuscan Archipelago is encompassed both by insular endemic taxa as

428 well as by widespread species (*A. agestis, C. pamphilus, C. alceae, Melitaea nevadensis, M.*

429 *jurtina, P. aegeria* and *Zerynthia cassandra*). While these latter species are currently treated

430 as being of 'Least Concern' in the European and Italian Red Lists (Van Swaay et al., 2010;

431   Balletto et al., 2015), some of them disappeared from some islands resulting in a loss of

432   faunistic and genetic diversity. Some of the populations that have apparently disappeared in

433   the last 10 years may be still rediscovered following dedicated field research, as occurred for

434   *Zerynthia cassandra*, discovered on Elba in 1932 but apparently disappeared before our

435   intensive collection effort (Appendix S1). Species that disappeared from at least one island

436   showed a higher overall population diversification (Dst) compared to persisting species. In

437   fact, our analyses revealed that species accumulated genetic diversification because of their

438   reduced migration and colonization capabilities; but these characteristics can also produce

439   higher extinction risk due to reduced gene flow and rescue effect. We showed that one of the

440   main peculiarities of Tuscan islands is the occurrence of strong genetic contrasts among

441   nearby areas. If an insular relict or a genetically endemic population goes extinct, it is likely

442   thatit would be replaced by conspecific propagules from the nearest source, thus lowering the

443   ancestral genetic diversification. There is also evidence for Mediterranean butterflies that the

444   presence of endemic and relict island populations limits the colonization by mainland

445   populations probably due to density-dependent phenomena (Dapporto et al. 2012, Vodă et al.

446   2015b). The establishment of the Tuscan Archipelago National Park in 1996 represented a

447   fundamental step for the broad-scale protection of island communities. Nevertheless, specific

448   conservation strategies tailored on particularly valuable species are still lacking because

449   evidence-based information are missing. Our integrated approach, by prioritizing species

450   according to their contribution to genetic diversity and by identifying the impact of ecological

451   drivers on the emergence and extinction of differentiated populations, allows conservation

452   priorities to be established, with a necessity for regular monitoring schemes. Such schemes

453   should evaluate population consistence and health, particularly focusing on population size

454   and trends, genetic load and persistence of habitat suitability.

455 Our study also suggested that reduction of shady areas might be a driver of species loss in the

456 Archipelago. Historically, wooded and shrub areas have been considerably reduced, mostly

457 on Capraia and Montecristo. It has been already reported that deforestation can be a main

458 driver for butterfly extinction in Mediterranean islands. For example, the strong reduction of

459 shady areas has been linked with post-glacial reduction of butterflies on Malta with the recent

460 extinction of most relict elements (Vodă et al., 2016). We thus indicate as a specific

461 conservation action to adopt environmental management procedures aimed at preserving and

462 favouring environmental heterogeneity, thus increasing resources availability and suitable

463 habitats for a larger number of butterfly species (Dennis, 2010). The increase of temperature

464 predicted by recent climate change scenarios suggests that environmental heterogeneity will

465 play a pivotal role in buffering increasing thermal and drought stress.

466 In conclusion, this study shows that the integration of molecular and trait data with long-term

467 occurrence records allows the identification of the eco-evolutionary processes underlying the

468 high butterfly diversity in this Mediterranean diversity hotspot (Dennis et al., 2008).

469 Evidence-based priorities for future conservation actions have been provided following the

470 theory of conservation biogeography (Ladle & Whittaker, 2011). Public institutions, such as

471 the NGO Legambiente and the Tuscan Archipelago National Park that have collaborated in

472 this project, have already used some of these results to raise awareness for island diversity

473 protection and to implement key conservation measures for butterflies.

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483

484 **Biosketch**

485 LD, RaV, AC, MM and RoV conceived the idea; LD, RaV, AC, MM, RoV, VD, LF, HB,

486 LPC, SS, FZ, UM and LV collected the data and the specimens in the study area; LD, RaV,

487 VD and RoV assessed the taxonomy of the study species; LD, MM, AC and SS compiled the

488 trait data; LD, VD, JCH, RaV, MM, AC, RoV, FZ, UM and LV managed and obtained the

489 COI sequences; LD, HB and EB gathered the occurrence data; LD, RaV, MM, JCH and AC

490 performed the data analysis; all the authors discussed the results and participated in writing

491 the paper.

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671   **Data accessibility.** The complete dataset, together with R scripts to replicate the analyses

672   published in the paper, is available in the Supporting Information. The COI sequences are also

673   available in the following repositories (Genbank, BOLD and IO databases, accession codes

674   for Genbank, XXXXX-XXXXX; BOLD, XXXXX).

675 **Table 1** Species traits used in the study with the description of the type of trait (sensu Moretti

676 et al. 2016), in bold and the relative functional hypothesis in italics; the trait(s) measured; a

677 description of the trait(s), the literature sources and the weights obtained by each trait in the

678 first two Principal Components (in the cases when the analysis has been carried out). PC1 and

679 PC2 represent the weights of the traits in the first two components obtained after principal

680 component analysis (PCA) of each type of trait. PCA on the single variable of host plants has

681 not been carried out and for wingspan only the first component returned an eigenvalue higher

682 than 1.

Type of trait	Functional hypothesis	Trait measured and description	Sources	PC1	PC2
Feeding	Species feeding on a larger number of plants have a wider niche, thus a higher potential to colonize islands (Dennis et al. 2012)	**Number of host plant genera** used by larvae as reported in two literature sources	Lafranchis (2007)	-	-
			Tolman & Lewington (2008)		
Morphology	Larger species are characterized by higher mobility (Sekar, 2012), thus more probabilities to cross sea barriers (Dennis et al. 2012)	**Wingspan** : mean between minimum and maximum size reported in four main sources for European butterflies. Tshikolovets (2011) reported size for males and females	Higgins & Riley (1970)	0.448	-
			Lafranchis (2000)	0.448	-
			Pamperis (2009)	0.446	-
			Tshikolovets (2011) males	0.448	-
			Tshikolovets (2011) females	0.448	-
Life history	Phenological attributes determine the length of the most mobile life stage of winged adults. The period of the year when it is expressed.	**Month of adult first emergence.** It ranges from the coldest, January (1), to the warmest, August (8). No butterfly species has a later first emergence in the	Authors' collection data	-0.293	-0.822

	These characteristics affect the possibility to cross sea barriers (Dapporto et al., 2012; Dennis et al. 2012) and can interact with climatic changes in determine extinction probabilities.	study area			
		Last month when adults fly, ranging from January (1) to December (12)	Authors' collection data	0.533	-0.482
		Length of the flight period: number of months when the adults occur in the study area	Authors' collection data	0.587	-0.189
		Voltinism. Number of generations/year in the study area	Authors' collection data and Tolman & Lewington (2008)	0.535	0.237
Physiology	Mean climatic conditions of the areas inhabited by a species are considered as good proxies for their ecophysiological response to climate (Devictor et al., 2012). They can affect the probability for species persistence in the warm and dry Mediterranean climate of Tuscan islands.	**Mean temperature** occurring in the 50×50 km spatial cells where the species has been modeled to occur	Schweiger et al. (2014)	0.334	-0.387
		Mean precipitation in the same spatial cells as above	Schweiger et al. (2014)	-0.292	-0.607
		Maximum temperature tolerance. Upper 95% confidence interval for temperature mean	Schweiger et al. (2014)	0.334	-0.387
		Minimum precipitation tolerance. Lower 95% confidence interval for precipitation mean	Schweiger et al. (2014)	-0.332	-0.413
		Overall temperature tolerance. Standard deviation for temperature mean	Schweiger et al. (2014)	-0.332	0.313
		Overall precipitation tolerance. Standard deviation for precipitation mean	Schweiger et al. (2014)	-0.337	-0.056

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Table 2 Species ranked for Gst, Dst and Uni, with information on single island uniqueness and documented recent extinction events. Dis, species that disappeared from Elba (E), Giglio (G) or Capraia (C). The last seven columns represent the uniqueness values of each species on each island. "-" means that the species has not been reported on that particular island, "NA" means that the species has been reported but that the population was not included in this study because of lack of genetic data. Taxa endemic to the insular region (Sardinia, Corsica and Tuscan islands) are written in bold, while the six species that were not recorded on islands during our surveys in the last 10 years are highlighted in grey.

Species	Gst	Dst	Uni	Dis	Gor	Cap	Elb	Pia	Mon	Gig	Gia
Aglais urticae/ichnusa	0.937	0.449	NA	E	-	-	NA	-	-	-	-
Anthocharis cardamines	0	0	0.051		NA	NA	0.051	NA	NA	NA	NA
Argynnis pandora	0.171	0.15	0.076		-	-	0.076	-	-	0.114	-
Argynnis paphia	NA	0	0	E	-	-	NA	-	-	-	-
Aricia agestis/cramera	0.738	0.984	0.094	G	-	0.152	0.095	0.046	-	NA	NA
Callophrys rubi	0.846	0.279	0		-	-	0	-	-	0	-
Carcharodus alceae	0.779	0.258	0.095		0	NA	0	0.456	NA	0.152	-
Celastrina argiolus	0.114	0.011	0.076		0	0	0.051	0	0	0.076	-
Charaxes jasius	0	0	0		NA	0	0	NA	-	0	0
Coenonympha corinna	0.943	2.194	0.19	G	-	0.456	0.101	-	-	NA	0
Coenonympha pamphilus	0.864	0.86	0.152		-	-	0.152	-	-	-	-
Colias croceus	NA	0	0		0	0	0	0	NA	0	NA
Euchloe ausonia	NA	0	0		-	-	0	-	-	-	-
Favonius quercus	0.393	0.053	0.051		-	-	0.152	-	-	-	-
Gegenes pumilio	NA	0	0		-	-	0	-	-	0	-
Glaucopsyche alexis	0	0	0		-	-	0	-	-	-	-
Gonepteryx cleopatra	0.385	0.004	0		0	0	0	0	-	0	0
Gonepteryx rhamni	0.323	0.237	NA	E	-	-	NA	-	-	-	-
Hipparchia aristaeus	0.119	0.019	NA	C	NA	NA	NA	-	-	NA	-
Hipparchia neomiris	0.871	0.968	End	C	-	NA	NA	-	-	-	-
Hipparchia statilinus	0.281	0.053	0		-	-	0	-	-	-	-
Iphiclides podalirius	0.701	0.063	0		-	-	0	-	-	-	NA
Issoria lathonia	0.431	0.014	0		-	-	0	-	-	0	-
Lampides boeticus	0.044	0.005	0.038		0	0.152	0	0	0	0	NA

Species										
Lasiommata megera/paramegaera	0.966	0.615	0.37		0	0.798	0.076	0	0.152	0	-
Leptidea sinapis	0.501	0.081	0	E	-	-	NA	-	-	-	-
Leptotes pirithous	0.298	0.048	0		-	0	0	NA	0	0	0
Limenitis reducta	0.185	0.015	0		-	-	0	-	-	-	-
Lycaena phlaeas	0.762	0.098	0.051	C	-	NA	0	0	0.152	0	-
Maniola jurtina	0.603	0.298	0.085	C	-	NA	0.092	0.076	-	0	-
Melitaea cinxia	0.379	0.54	NA	E	-	-	NA	-	-	-	-
Melitaea didyma	0.344	0.07	0		-	-	0	-	-	-	-
Melitaea nevadensis	0.455	0.492	0.988		-	-	0.988	-	-	-	-
Nymphalis polychloros	0.415	0.043	NA	E	-	-	NA	-	-	-	-
Papilio machaon	0	0	0.038		0	NA	0	0	-	0.152	-
Pararge aegeria	0.88	0.636	0.076		NA	0.076	NA	0.076	NA	NA	NA
Pieris brassicae	0	0	0.076		NA	NA	0	0	NA	0.101	NA
Pieris mannii	0	0	0		-	-	0	-	-	-	-
Pieris napi	0.432	0.133	0		NA	-	0	-	-	-	0
Pieris rapae	0.118	0.05	0.054		0.157	0.051	0	0.038	NA	0	0
Plebejus bellieri/idas	0.616	0.134	0.051		-	-	0.152	-	-	-	-
Polyommatus icarus	0.216	0.018	0.076	G	-	0.076	0	0	-	NA	-
Pontia edusa	0.079	0.015	0.051	C	-	*	0.076	NA	-	0	NA
Pyrgus armoricanus	NA	0	0		-	-	0	-	-	-	-
Pyronia cecilia	0.26	0.224	0.091	C	-	NA	0	0.152	-	0.076	-
Pyronia tithonus	0.26	0.015	0		-	-	0	-	-	-	-
Satyrium ilicis	NA	0	0		-	-	0	-	-	-	-
Spialia sertorius	0.417	0.009	0		-	-	0	-	-	-	-
Thymelicus acteon	0.432	0.067	0		-	-	0	-	-	-	-
Vanessa atalanta	0.127	0.009	0.051		NA	0.051	0	NA	0	0	NA
Vanessa cardui	0.032	0.007	0.038		0	0	0.076	0	NA	0	0.076
Zerynthia cassandra	0.293	0.053	0.152		-	-	0.228	-	-	-	-

698

699

700

701 **Table 3** Trait variables entered in the AIC phylogenetic regressions for Gst, Dst and

702 Uniqueness (Uni). And the differences in Gst, Dst and Uniqueness between species that have

703 disappeared at least from one island, compared to species that have not disappeared

	Trait variables	Estimate	StdErr	t.value	p.value
Gst	Host Plants	-0.100	0.045	-2.226	0.032
	Mobility PC1	-0.141	0.046	-3.031	0.004
	Phenol PC1	0.058	0.043	1.363	0.181
	Ecophy PC2	0.068	0.040	1.713	0.095
Dst	Host Plants	-0.110	0.043	-2.550	0.015
	MobilityPC1	-0.126	0.047	-2.660	0.011
	Phenol PC1	0.060	0.042	1.454	0.153
	Ecophy PC2	0.119	0.039	3.042	0.004
Uni	Host Plants	-0.045	0.028	-1.643	0.108
	Ecophy PC2	0.102	0.033	3.112	0.003

704

705 **Table 4** The effects of trait variables in the logistic phylogenetic regressions for species

706 disappearance in Elba and Capraia.

Island	PC1/PC2	Estimate	StdErr	z.value	p.value
Elba	Host_Plants	-0.380	0.469	-0.810	0.418
	Mobility PC1	0.412	0.482	0.855	0.393
	Phenol PC1	-0.382	0.544	-0.702	0.483
	Phenol PC2	-0.522	0.437	-1.194	0.233
	Ecophy PC1	-0.813	0.584	-1.393	0.164
	Ecophy PC2	0.766	0.561	1.367	0.172
Capraia	Host_Plants	-0.969	0.972	-0.997	0.319
	Mobility PC1	1.605	1.206	1.331	0.183
	Phenol PC1	-6.375	3.002	-2.124	0.034
	Phenol PC2	2.835	1.869	1.517	0.129
	Ecophy PC1	-1.820	1.196	-1.522	0.128
	Ecophy PC2	2.753	1.783	1.544	0.123

707

708

709 **Figure 1** (a) The study region where the islands and mainland areas are highlighted with the

710 same colours obtained in the RGB projection of the Principal Coordinate Analyses (PCoA) in

711 figure 3; (b) the workflow of the protocol used for the analyses.

712
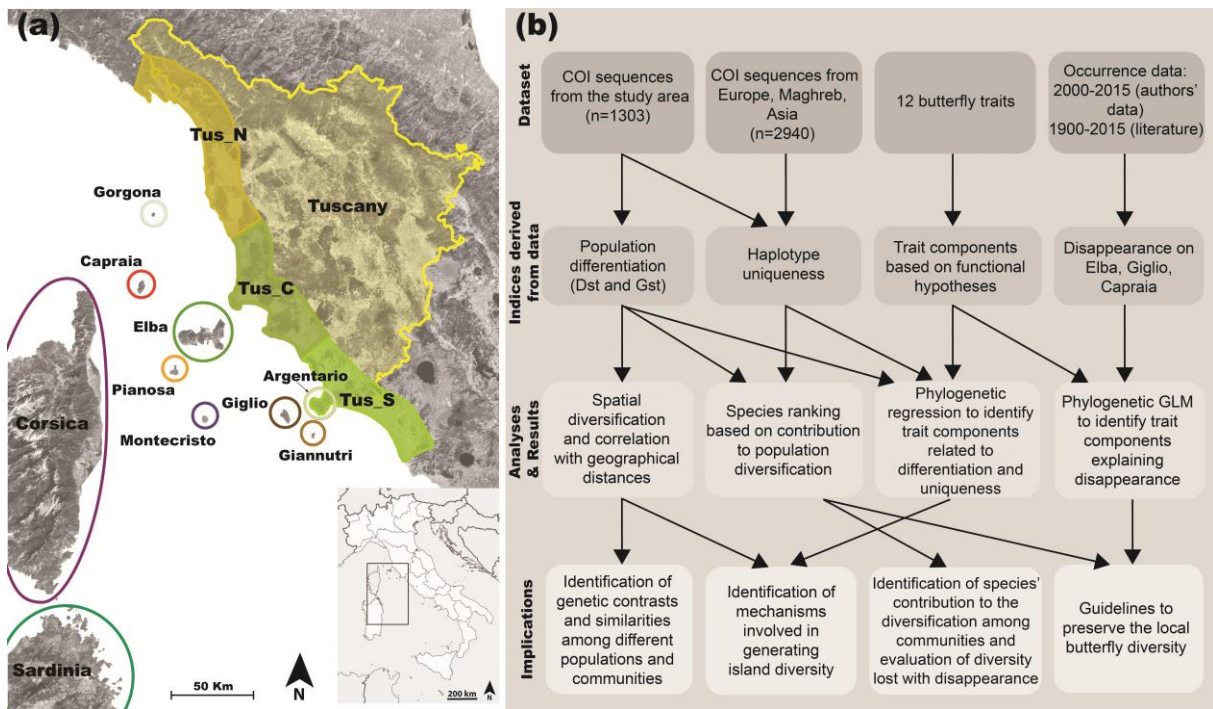
713

714

715   **Figure 2** Phylogenetic tree based on cytochrome *c* oxidase subunit 1 (COI) sequences of the

716   butterfly species occurring in the Tuscan Archipelago, with their Gst, Dst, Uni and ecological

717   traits. Square root Dst values are mapped over the tree, while Gst and Uni are reported as

718   coloured squares (character mapping on the tree for Gst and Uni is available in Appendix S1).

719   Host plants represent the number of plant genera on which the larva of a given species has

720   been reported: one leaf - one genus; two leaves two to four genera, three leaves more than

721   four genera. The sizes of the butterfly silhouettes are directly correlated with the species size

722   (wingspan). Ecophy 1 represents the first PC of physiology traits mostly represent mean

723   temperature and is reported with colours representing quartiles of values (red, preference for

724   warm temperatures; blue, preference for cold temperatures). Ecophy 2 represents the second

725   PC of physiology traits mostly correlated with precipitation tolerance and represented in

726   quartile from small (high tolerance to drought) to large drops (low tolerance to drought).

727   Phenol 1 represent quartiles the first PC of phenology correlated with the length of flying

728   period from shortest (one black sector) to longest (four black sectors). Phenol 2, mostly linked

729   to the period of emergence, represent spring species appearing early in the year (black sector

730   right-top) to species with a later summer-autumn appearance (black sector left-top).

731

732

733

734
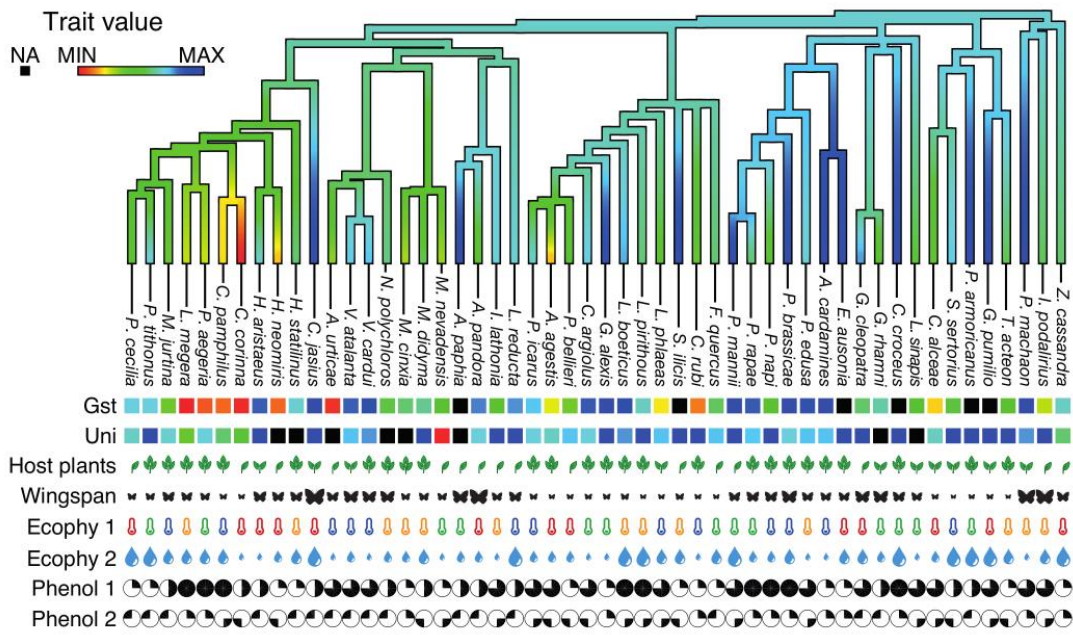
Figure 3 Overall genetic patterns obtained after comparisons among islands based on Gst. (a)

The colours obtained in the RGB projection of the Principal Coordinate Analyses (PCoA) are

more similar among more genetically similar island communities. When the colours are

reported on a map (b) of the studied region they show similarity and contrast among areas.

The locality codes are: Arg, Argentario; Cap, Capraia; Cor, Corsica; Elb, Elba; Gia,

Giannutri; Gig, Giglio; Gor, Gorgona; Mon, Montecristo; Pia, Pianosa; Sar, Sardinia; Tus,

Tuscany; T_C central Tuscany coast; T_N, northern Tuscany coast; T_S, southern Tuscany

coast. (c) Haplotype networks based on the COI gene for nine species that exemplify different

patterns of genetic variation, obtained by comparing haplotypes from the study area with

other regions of Europe (c). A molecular assessment for all the species is available in the

Supporting Information (see Appendix S1).

746

747
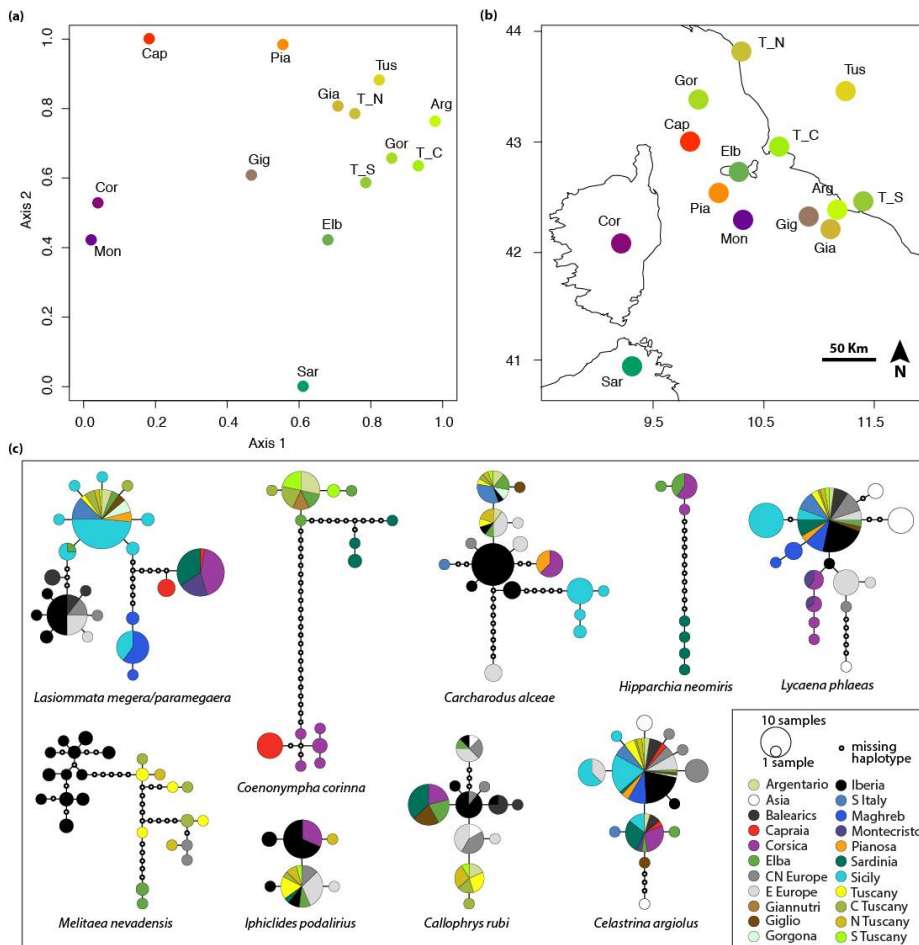
Supporting Information

Additional Supporting Information is available in the online version of this study:

Appendix S1 {Supplementary methods and results containing the taxonomic assessment for each species, the occurrence data on Tuscan islands from 1900 to 2015 and the COI assessment for all the species. Supplementary results for PCA and Phylogenetic Models are also provided}

755   Appendix S2 {The dataset in the form of a fasta file (sequences_TA.fas), the information

756   about the specimens used in the study (dataset.txt), the table containing the

757   ecological traits for the 52 species (selected.traits.txt) and the R scripts used to

758   carry out the analyses (Dapporto_et_al_script.R)}

759

760   **Conflict of Interest:**

761   The authors declare no conflicts of interest

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775

776 **Appendix S1**

777

778

779 **Rise and fall of island butterfly diversity. Understanding population diversification and**
780 **extinction in a highly diverse Archipelago.**

781

782 Dapporto L., Cini A., Menchetti M., Vodă R., Bonelli S, Casacci L.P., Dincă V., Scalercio S.,
783 Hinojosa J.C., Biermann H., Forbicioni L., Mazzantini U., Venturi L., Zanichelli F., Balletto E.,
784 Shreeve T.G., Dennis R.L.H., Vila R.

785

786

787

788   Calculation of Dst and Gst indexes (Nei, 1987).

789

790   Dst is defined as:

791   Dst = Ht - Hs

792   where Ht represents the average intraspecific p-distances for all specimens of a given
793   species, and Hs is the average of the intra-population p-distances. Thus, Dst represents the
794   average genetic differentiation among populations in p-distance units.

795

796   The second measure (Gst) is a standardized index (Nei, 1987) defined as:

797   Gst = Dst/Ht

798   This index ranges from negative values to 1 (complete differentiation). Negative values
799   (intra-area differentiation higher than inter-area differentiation) can have different subtle
800   meanings, but most often are a bias due to relatively small sample sizes; usually they are set
801   to zero (Meirmans & Hedrick, 2011) and for several cases in our study we set the negative
802   values to zero.

803

804   We also calculated the pairwise Gst among all pairs of populations for each species, using
805   the following formula:

806    $Gst_{i,j} = Dst_{i,j}/Ht$

807   This represents the specific fraction of the overall genetic diversity (Ht) expressed as the
808   inter-population diversification ($Dst_{i,j}$) between a given pair of areas (i and j).

809

810

811   In the following section distributional, taxonomic and historical notes for all the species are
812   reported together with their assessment for population diversification. The genetic distances
813   among haplotypes have been reduced to two dimension by using Principal Component
814   Analysis. The resulting configuration has been plotted in RGB space then the resulting
815   colours for specimens reported in the map with pie charts.

816

817   *Carcharodus alceae* (Esper, 1780)

818

819   **1900-2005**

820   Elba: 1908, 1916, 1921, 1951-1961, 1963, 1980 (Biermann & Hesch, 1982 , Balletto et al.,
821   2007), 2000-2003 (authors).

822   Giglio: 1980 (Biermann & Hesch, 1982).

823   Pianosa: 1998 (Dapporto et al. 1999).

824   **2006-2015**

825   Elba: 2008-2015 (authors).

826   Giglio: 2010, 2013, 2014 (authors).

827   Capraia: 2012 (authors).

828   Gorgona: 2015 (authors).

829

830   This species showed some diversification among haplotypes (maximum p-distance 0.9%) and
831   a relatively high population diversification (Dst 0.258 and Gst 0.779). Some haplotypes from
832   Pianosa and Giglio were not found on the mainland resulting in a uniqueness of 0.095.
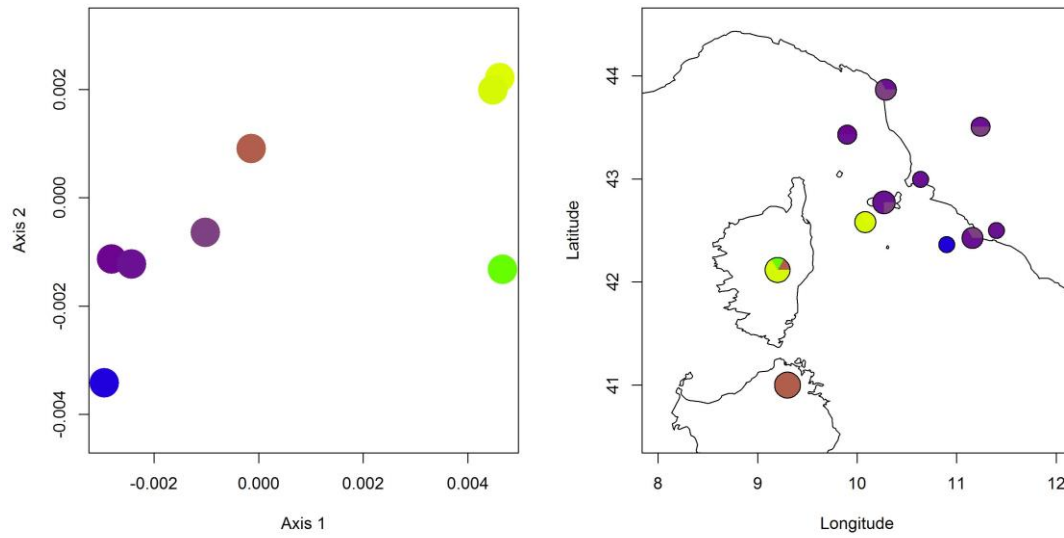
833

834

835 Figure S1. PCoA projection (left) of genetic distances among specimens (dots) in the
836 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
837 map (right). The first two PCoA axes explained 99.43% of variance.

838

839   *Spialia sertorius* (Hoffmannsegg, 1804)

840

841   The taxon living in Sardinia and Corsica was identified in the past as *Spialia sertorius*
842   *therapne*. Recent molecular studies showed that *S. therapne* must be considered as a good
843   species with a diversification higher than 3% respect to *S. sertorius* (Hernández-Roldán et al,
844   2016). For this reason we did not include Sardo-Corsican populations of *S. therapne* as
845   comparison for *S. sertorius*.

846

847   **1900-2005**

848   Elba: 1908, 1916, 1921, 1963 (Biermann & Hesch, 1982 , Balletto et al., 2007).

849   **2006-2015**

850   Elba: 2009, 2010, 2013, 2015 (authors).

851

852   This species showed a low diversification among haplotypes (maximum p-distance 0.2%),
853   and a rather low population diversification (Dst 0.009 and Gst 0.417). All the haplotypes
854   found on Elba island were also found on the mainland.

855
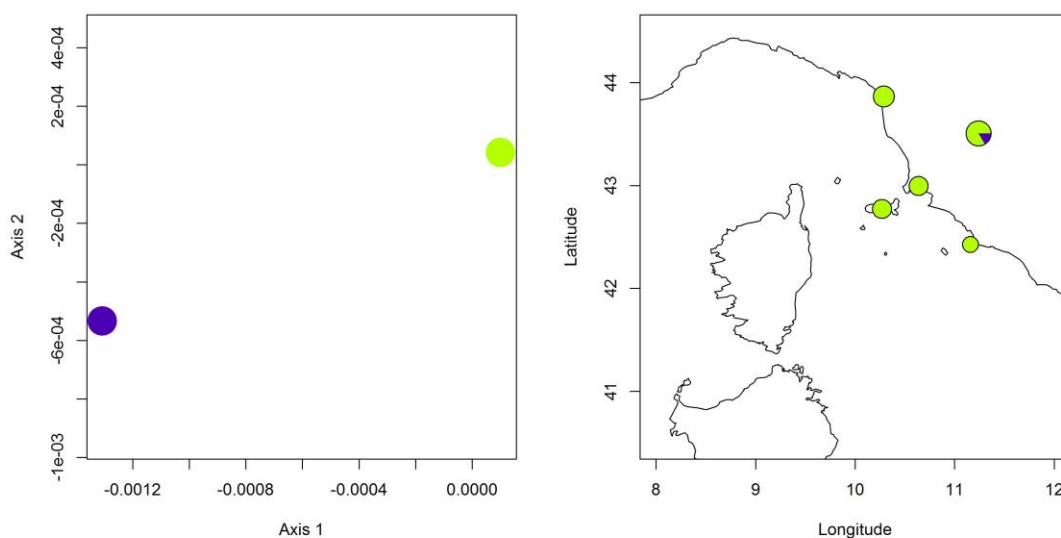
856

857

858   Figure S2. PCoA projection (left) of genetic distances among specimens (dots) in the
859   bidimensional RGB colour space. Individual colours have been subsequently plotted on the
860   map (right). The first two PCoA axes explained 100.00% of variance.

861

862 *Pyrgus armoricanus* (Oberthür, 1910)

863

864 **1900-2005**

865 Elba: 1916, 1956, 1957, 1963 (Biermann & Hesch, 1982 , Balletto et al., 2007).

866 **2006-2015**

867 Elba: 2013 (authors).

868

869 This species showed a single haplotype in the 13 specimens we analyzed from Tuscany, Elba
870 and Corsica.

871

872 *Thymelicus acteon* (von Rottemburg, 1775)

873

874 **1900-2005**

875 Elba: 1908, 1916, 1921, 1963, 1976 (Biermann & Hesch, 1982 , Balletto et al., 2007), 2000-
876 2004 (authors).

877 **2006-2015**

878 Elba: 2008, 2010, 2012, 2015 (authors).

879

880 This species showed a low diversification among haplotypes (maximum p-distance 0.5%),
881 and a medium population diversification (Dst 0.067 and Gst 0.432) mostly due to the
882 occurrence on Elba of a single haplotype very uncommon on the Tuscan mainland.
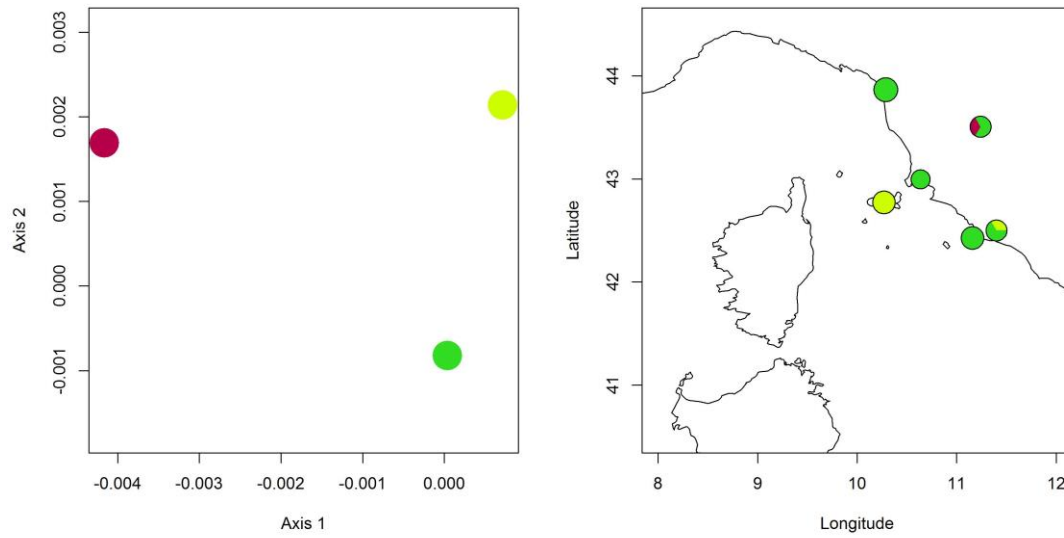
883

884

885 Figure S3.   PCoA projection (left) of genetic distances among specimens (dots) in the
886 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
887 map (right). The first two PCoA axes explained 100% of variance.

888

889

Gegenes pumilio (Hoffmannsegg, 1804)

1900-2005

Elba: 1916, 1951, 1956, 1957, 1963, 1976 (Biermann & Hesch, 1982 , Balletto et al., 2007), 2000 (authors).

2006-2015

Elba: 2008, 2013, 2015 (authors).

Giglio: 2013 (authors).

This species showed a single haplotype in the 5 specimens we analyzed from Tuscany, Elba, Giglio and Sardinia.

Zerynthia cassandra (Geyer, 1828)

1900-2005

Elba: 1932 (Bryk, 1932).

2006-2015

Elba: 2008-2011, 2013, 2014 (authors).

This species showed some diversification among haplotypes (maximum p-distance 0.8%), and a rather low population diversification (Dst 0.053 and Gst 0.293) mostly due to the occurrence on Elba of a single haplotype not occurring on the Tuscan mainland. This haplotype also produced a uniqueness of 0.152.
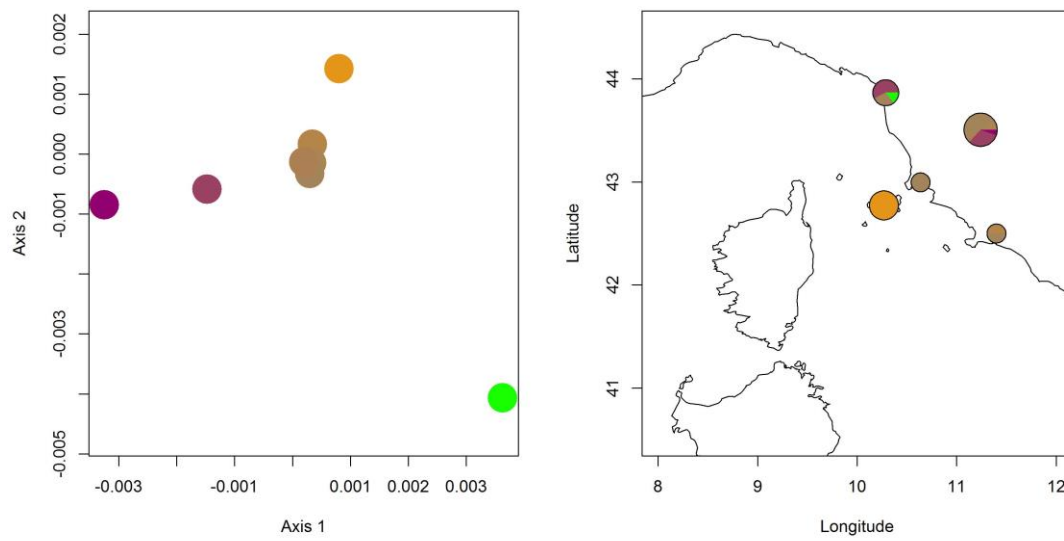
914

915

916 Figure S4. PCoA projection (left) of genetic distances among specimens (dots) in the
917 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
918 map (right). The first two PCoA axes explained 73.84% of variance.

919

920   *Iphiclides podalirius* (Linnaeus, 1758)

921

922   **1900-2005**

923   Elba: 1908, 1916, 1921, 1963, 1976 (Biermann & Hesch, 1982 , Balletto et al., 2007), 1998
924   (Biermann, 1999).

925   Giglio: no collection year (Balletto et al. 2007).

926   **2006-2015**

927   Elba: 2014-2015 (authors).

928

929   This species showed a low diversification among haplotypes (maximum p-distance 0.3%),
930   and a low Dst (0.063). Nevertheless it showed a high Gst of 0.701, mostly due to the
931   occurrence on Corsica of a single haplotype not occurring in the rest of the study area. All
932   the island haplotypes have been found in other areas of the European mainland.

933
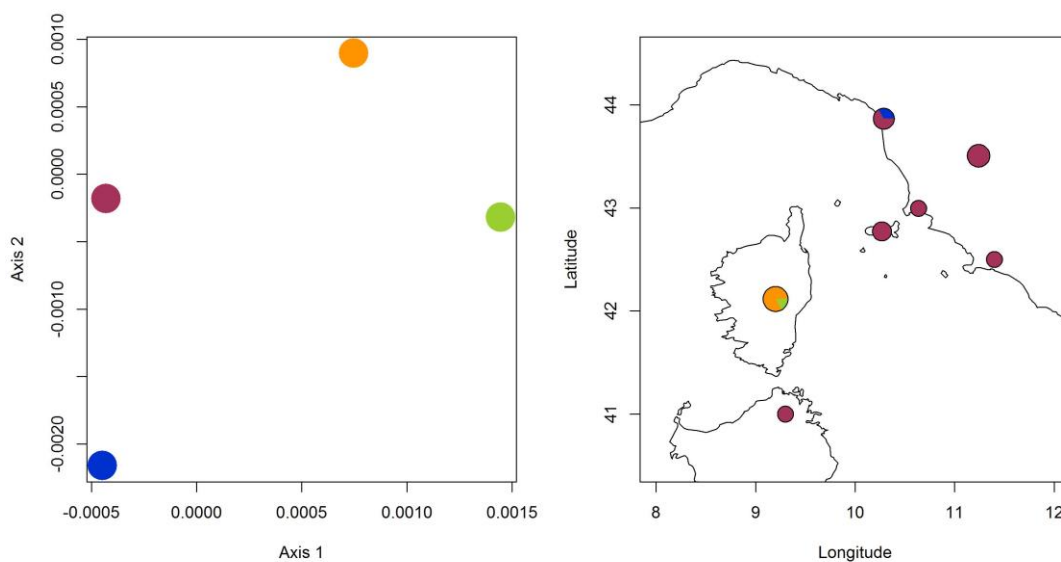
934

935

936   Figure S5.   PCoA projection (left) of genetic distances among specimens (dots) in the
937   bidimensional RGB colour space. Individual colours have been subsequently plotted on the
938   map (right). The first two PCoA axes explained 100% of variance.

939

940

941   *Papilio machaon* Linnaeus, 1758

942

943   **1900-2005**

944   Capraia: 1914 (Razzauti, 1917).

945   Elba: 1908, 1916, 1921, 1963, 1976, 1980 (Biermann & Hesch, 1982 , Balletto et al., 2007),
946   1998 (Biermann, 1999), 2000 (authors).

947   Pianosa: 1998 (Dapporto et al. 1999).

948   Gorgona: 1999-2002 (Dapporto, 2002).

949   **2006-2015**

950   Elba: 2009-2011, 2013-2015 (authors).

951   Giglio: 2007, 2010-2014 (authors).

952   Gorgona: 2015 (authors).

953   Pianosa: 2014 (authors).

954

955   *Not recorded on Capraia in the last 10 years.*

956

957   This species showed a high diversification among haplotypes (maximum p-distance 1.7%),
958   but their distribution was not spatially structured resulting in no population diversification
959   (Dst and Gst 0). The detection on Giglio of a haplotype not recorded on the mainland
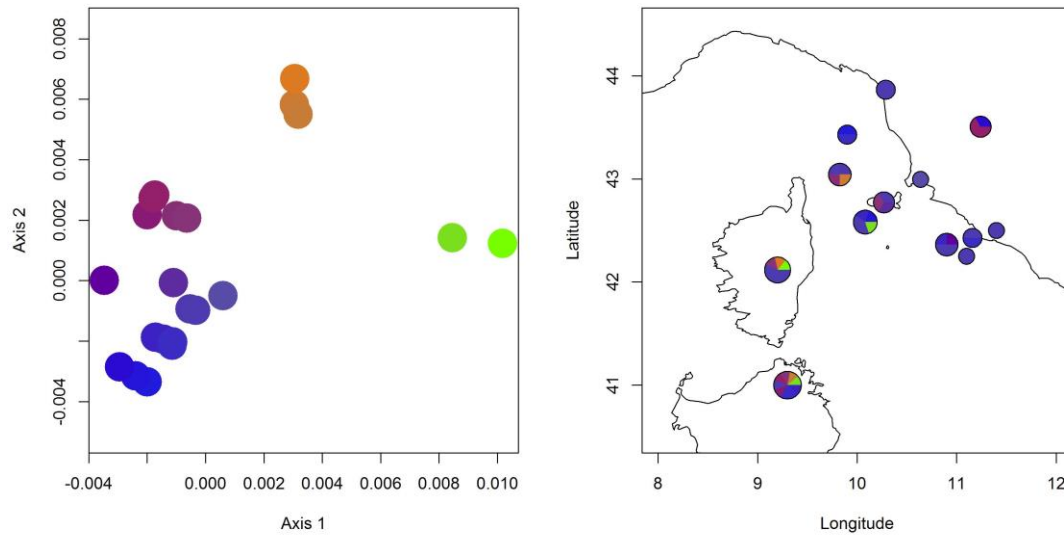960   resulted in a uniqueness value of 0.038.

961
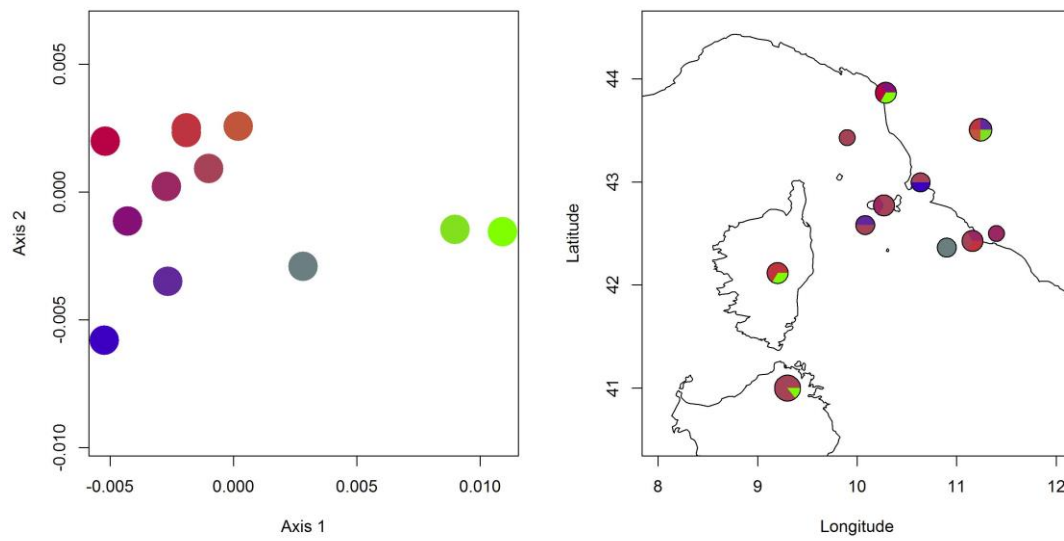
962

963

964 Figure S6.   PCoA projection (left) of genetic distances among specimens (dots) in the
965 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
966 map (right). The first two PCoA axes explained 89.53% of variance.

967

968 *Pieris brassicae* (Linnaeus, 1758)

969

970 **1900-2005**

971 Gorgona: 1999-2002 (Dapporto, 2004).

972 Capraia: 1915 (Razzauti, 1917), 2000 (authors).

973 Elba: 1908, 1916, 1921, 1963 (Biermann & Hesch, 1982 , Balletto et al., 2007), 1998
974 (Biermann, 1999), 2000-2003 (authors).

975 Montecristo: 2001 (authors).

976 **2006-2015**

977 Elba: 2008-2015 (authors).

978 Giglio: 2007, 2010, 2013 (authors).

979 Capraia: 2006 (authors).

980 Pianosa: 2008, 2014 (authors).

981

982 This species showed a diversification among haplotypes (maximum p-distance 0.9%), but
983 their distribution was not spatially structured resulting in no population diversification (Dst
984 and Gst 0). The detection on Giglio island of a haplotype not found on the mainland resulted
985 in a uniqueness value of 0.076.

986
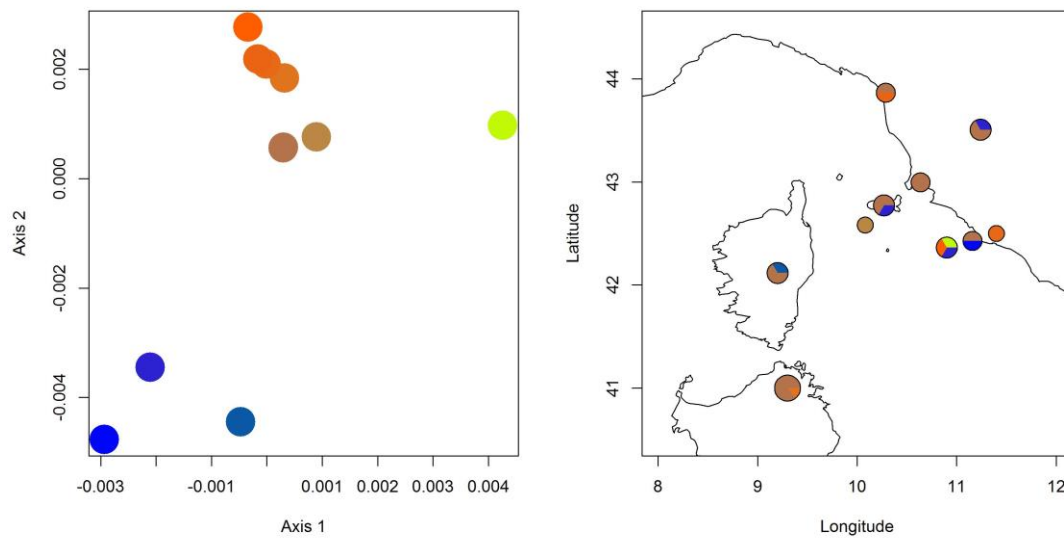
987

988

989   Figure S7.   PCoA projection (left) of genetic distances among specimens (dots) in the
990   bidimensional RGB colour space. Individual colours have been subsequently plotted on the
991   map (right). The first two PCoA axes explained 92.72% of variance.

992

993   *Pieris mannii* (Mayer, 1851)

994

995   **1900-2005**

996   Elba: 1908, 1915, 1921, 1956, 1963, 1963 (Biermann & Hesch, 1982 , Balletto et al., 2007).

997   Gorgona: 1999-2002 (Dapporto, 2004).

998   **2006-2015**

999   Elba: 2008-2010, 2012 (authors).

1000

1001   This species is uncommon in the study area and we only examined five specimens. They
1002   showed a very low diversification among haplotypes (maximum p-distance 0.5%), and their
1003   distribution was not spatially structured resulting in no population diversification (Dst and
1004   Gst 0). The haplotype found on Elba was also found on the mainland.

1005
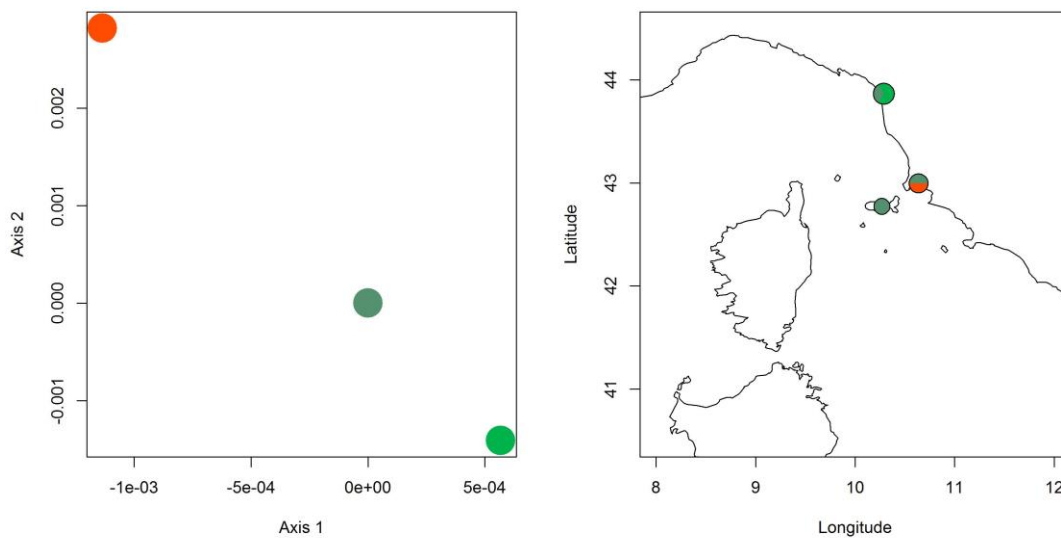
1006

1007

1008   Figure S8. PCoA projection (left) of genetic distances among specimens (dots) in the
1009   bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1010   map (right). The first two PCoA axes explained 100% of variance.

1011

1012 *Pieris rapae* (Linnaeus, 1758)

1013

1014 **1900-2005**

1015 Elba: 1908, 1916, 1921, 1963, 1980 (Biermann & Hesch, 1982 , Balletto et al., 2007), 1998
1016 (Biermann, 1999), 2000-2003 (authors).

1017 Giglio: 1908 (Rocci and Turati, 1925), 1980 (Biermann & Hesch, 1982), 2003, 2004 (authors).

1018 Gorgona: 1999-2002 (Dapporto, 2004).

1019 Capraia: 1915 (Razzauti, 1917), 1980 (Biermann & Hesch, 1982), 2000-2003 (authors).

1020 Pianosa: 1998 (Dapporto et al. 1999).

1021 Montecristo: 1983 (Raineri, 1986).

1022 **2006-2015**

1023 Elba: 2008-2015 (authors).

1024 Giglio: 2007, 2010, 2013, 2014 (authors).

1025 Gorgona: 2015 (authors).

1026 Capraia: 2006-2008, 2010, 2013, 2014 (authors).

1027 Giannutri: 2014 (authors).

1028 Pianosa: 2011, 2013 (authors).

1029

1030 This species showed a high diversification among haplotypes (maximum p-distance 1.4%),
1031 but their distribution was not spatially structured resulting in low population diversification
1032 (Dst 0.050 and Gst 0.112). The detection on Tuscan islands of haplotypes not found on the
1033 mainland resulted in a uniqueness value of 0.054.

1034

Figure S9. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 75.52% of variance.

1042 *Pieris napi* (Linnaeus, 1758)

1043

1044 **1900-2005**

1045 Elba: 1908, 1916, 1921, 1979, 1980 (Biermann & Hesch, 1982 , Balletto et al., 2007), 1998
1046 (Biermann, 1999), 2000-2003 (authors).

1047 Gorgona: 1999-2002 (Dapporto, 2004).

1048 **2006-2015**

1049 Elba: 2008-2011, 2015 (authors).

1050 Giannutri: 2014 (authors).

1051

1052 This species showed a diversification among haplotypes (maximum p-distance 1.1%), and a
1053 discrete population diversification (Dst 0.133 and Gst 0.432) mostly due to the occurrence
1054 on Corsica of a series of haplotypes uncommon on the Tuscan mainland, Elba and Giannutri.
1055 All island haplotypes have been also found on the mainland.

1056
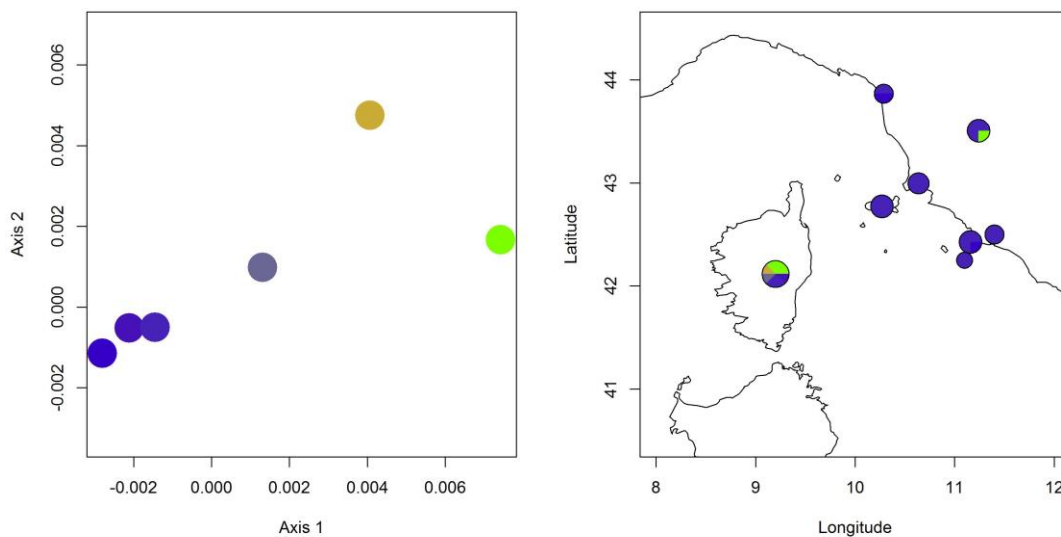
1057

1058

1059   Figure S10.   PCoA projection (left) of genetic distances among specimens (dots) in the

1060   bidimensional RGB colour space. Individual colours have been subsequently plotted on the

1061   map (right). The first two PCoA axes explained 98.41% of variance.

1062

1063

1064 *Pontia edusa* (Fabricius, 1777)

1065

1066 It has been recognized since decades that *Pontia edusa* and *Pontia daplidice* represent two
1067 highly differentiated cryptic species showing 7% of COI divergence and differences in
1068 allozymes (revised by John et al. 2013). For this reason, they have been considered as
1069 distinct entities in this study. In Sardinia and Corsica only *P. daplidice* has been found, while
1070 on Tuscan islands only *P. edusa* has been identified so far. It is unknown which species was
1071 found in Capraia between 1968 and 1970 (Gross, 1970). We attributed the population to *P.*
1072 *edusa* for the extinction analysis and it is unlikely that this decision have affected the results
1073 of the phylogenetic regression since the traits of the two species are almost identical.

1074

1075 **1900-2005**

1076 Elba: 1908, 1916, 1921, 1963, 1980 (Biermann & Hesch, 1982 , Balletto et al., 2007).

1077 Pianosa: 1998 (Dapporto et al. 1999), 2000, 2003 (authors).

1078 Giglio: 1908 (Rocci & Turati, 1908).

1079 Capraia: 1968-1970 (Gross, 1970).

1080 **2006-2015**

1081 Elba: 2008-2013 (authors).

1082 Giglio: 2007, 2010, 2013, 2014 (authors).

1083

1084 *Not recorded on Capraia in the last 10 years.*

1085

1086 This species showed some diversification among haplotypes (maximum p-distance 0.8%),
1087 but their distribution was not spatially structured resulting in low population diversification
1088 (Dst 0.015 and Gst 0.079). The detection on Elba of a haplotype not found on the mainland
1089 resulted in a uniqueness value of 0.051.

1090
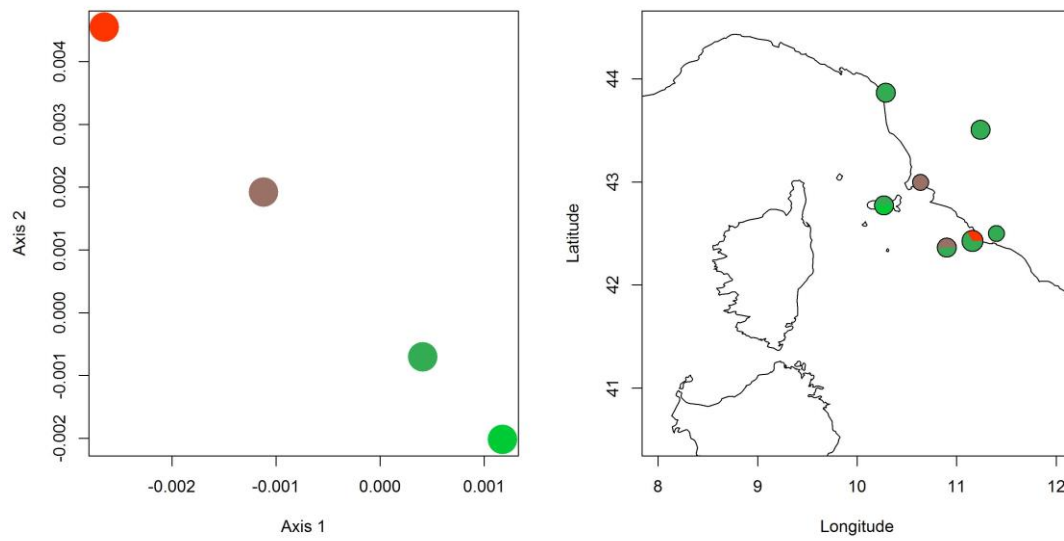
1091

1092

1093 Figure S11. PCoA projection (left) of genetic distances among specimens (dots) in the
1094 bidimensional RGB color space. Individual colours have been subsequently plotted on the
1095 map (right). The first two PCoA axes explained 100% of variance.

1096

1097 *Euchloë ausonia* (Hübner, 1804)

1098

1099 In Sardinia and Corsica this species is replaced by the endemic taxon *E. insularis*. *E. ausonia*
1100 and *E. insularis* are not sister species and their p-distance in COI sequences is about 5%
1101 (unpublished data). For this reason, we did not include *E. insularis* in the analysis.

1102

1103 **1900-2005**

1104 Elba: 1917 (Biermann & Hesch, 1982 , Balletto et al., 2007), 1997, 1998 (Biermann, 1999).

1105 **2006-2015**

1106 Elba: 2008, 2009, 2013, 2014 (authors).

1107

1108 This species showed a single haplotype in the 13 specimens we analyzed from Tuscany,
1109 Argentario and Elba.

1110

1111

1112 *Anthocharis cardamines* (Linnaeus, 1758)

1113

1114 **1900-2005**

1115 Elba: 1998 (Biermann, 1999).

1116 **2006-2015**

1117 Elba: 2008, 2010, 2011, 2013, 2014 (authors).

1118

1119 This species showed a low diversification among haplotypes (maximum p-distance 0.5%),
1120 and their distribution was not spatially structured resulting in no population diversification
1121 (Dst and Gst 0). The detection on Elba of a haplotypes not found on the mainland resulted in
1122 a uniqueness value of 0.051.

1123
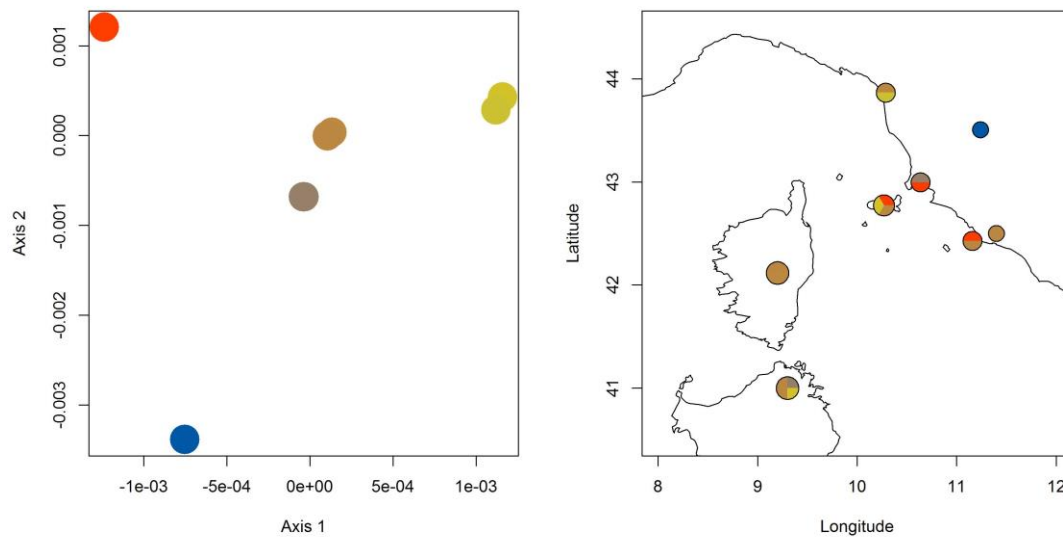
Figure S12. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 73.44% of variance.

Colias croceus (Geoffroy, 1785)

1900-2005

Elba: 1908, 1916, 1921, 1963, 1976, 1980 (Biermann & Hesch, 1982 , Balletto et al., 2007), 1998 (Biermann, 1999), 2000-2003 (authors).

Capraia: 1968-1970 (Gross, 1970), 1996 (Jutzeler & de Bros, 1997), 2000 (authors).

Giglio: 1980 (Biermann & Hesch, 1982), 2003 (authors).

Montecristo: 1983 (Raineri, 1986).

Gorgona: 1999-2002 (Dapporto, 2004)

Pianosa: 1998 (Dapporto et al. 1999).

2006-2016

1142   Elba:, 2008-2015 (authors).

1143   Giglio: 2007, 2010, 2012-2014 (authors).

1144   Gorgona: 2015 (authors).

1145   Capraia: 2007, 2008, 2010, 2012, 2014 (authors).

1146   Pianosa: 2011, 2014 (authors).

1147

1148   This species showed a single haplotype in the 32 specimens we analyzed from several areas.

1149

1150

1151 *Gonepteryx cleopatra* (Linnaeus, 1767)

1152

1153 **1900-2005**

1154 Elba: 1908, 1916, 1921, 1963, 1976, 1980 (Biermann & Hesch, 1982 , Balletto et al., 2007),
1155 2000, 2003 (authors).

1156 Capraia: 1914 (Razzauti, 1917), 1978 (Biermann & Hesch, 1982), 1996 (Jutzeler & de Bros,
1157 1998), 2002 (authors).

1158 Giglio: 1980 (Biermann & Hesch, 1982).

1159 **2006-2015**

1160 Elba: 2008-2010, 2012-2015 (authors).

1161 Giglio: 2007, 2008, 2010, 2012, 2013 (authors).

1162 Giannutri: 2014 (authors).

1163 Gorgona: 2015 (authors).

1164 Capraia: 2008, 2010, 2012-2014 (authors).

1165 Pianosa: 2011, 2014 (authors).

1166

1167 This species showed only two haplotypes differentiated for a single mutation in the 32
1168 specimens we analyzed from several areas. One of the two haplotypes has only been found
1169 on Sardinia and on other mainland regions outside the study area. This resulted in some Gst
1170 (0.385) in a low Dst (0.004) and in a zero value for Uni.

1171
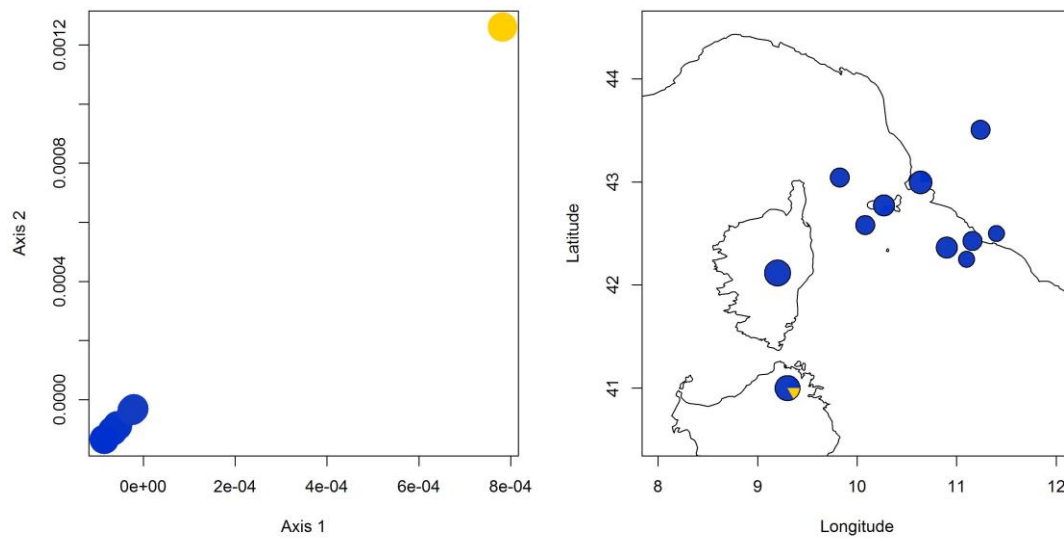
1172

1173

1174 Figure S13. PCoA projection (left) of genetic distances among specimens (dots) in the
1175 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1176 map (right). The first two PCoA axes explained 100% of variance.

1177

1178

1179   *Gonepteryx rhamni* (Linnaeus, 1758)

1180

1181   **1900-2005**

1182   Elba: 1908 (Biermann & Hesch, 1982 , Balletto et al., 2007).

1183

1184   In the study area this species showed a series of haplotypes with a high diversification
1185   (maximum p-distance 1.4%). However, they revealed to be only partially spatially structured
1186   resulting in low values of population differentiation (Dst 0.237, Gst 0.323).

1187

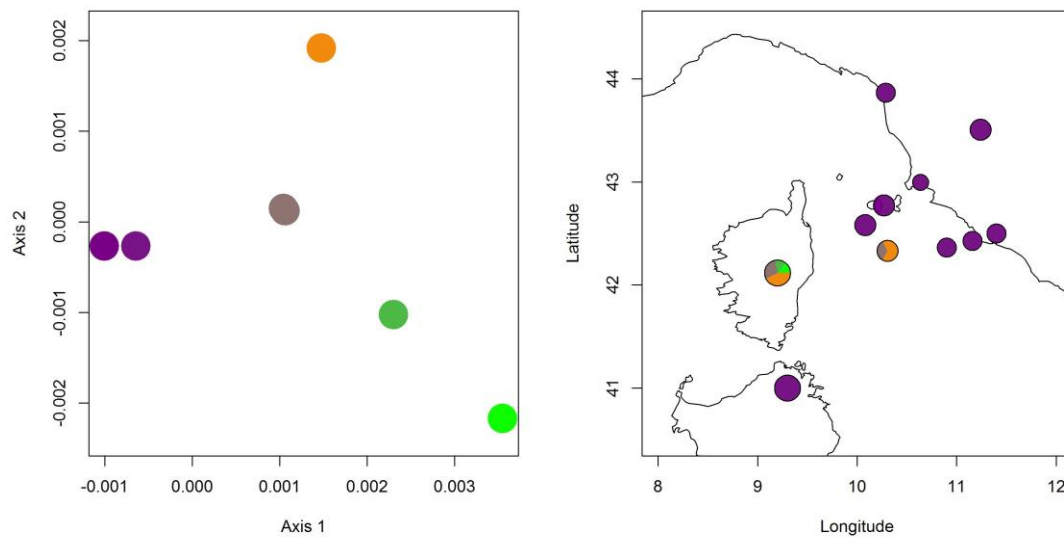1188   *Not recorded on Elba in the last 10 years.*

1189
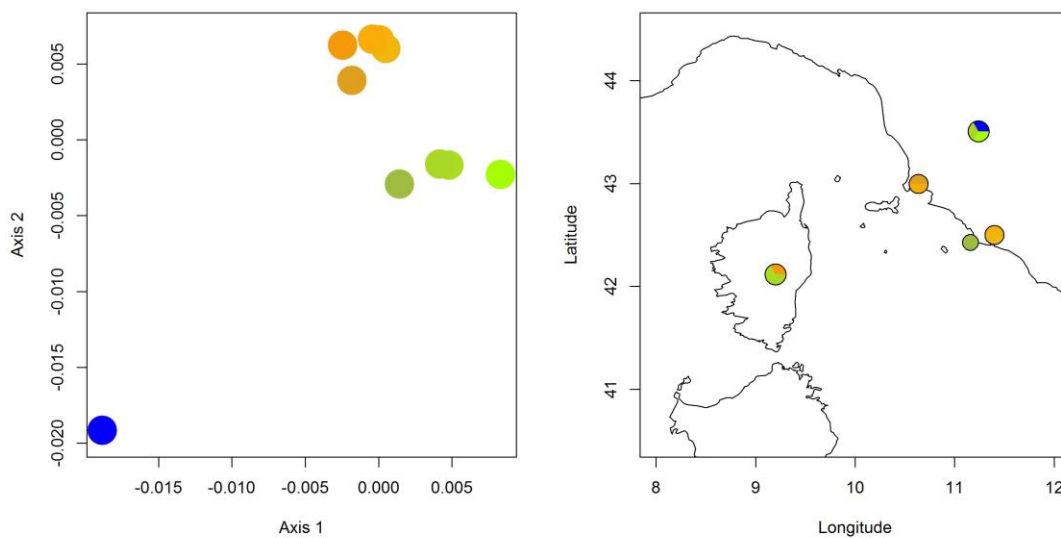
1190

1191

1192   Figure S14.   PCoA projection (left) of genetic distances among specimens (dots) in the
1193   bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1194   map (right). The first two PCoA axes explained 87.53% of variance.

1195

1196 *Leptidea sinapis* (Linnaeus, 1758)

1197

1198 It has been recently showed that in Europe the sinapis group is composed by a triplet of
1199 species (*L. sinapis*, *L. reali* and *L. juvernica*). Over the study area (Sardinia, Corsica, Tuscan
1200 islands and Tuscany mainland), only *L. sinapis* has been found so far. The species was
1201 probably common on Elba island in the past and a large series of specimens is preserved in
1202 the Roger Verity collection in MZUF. The last report for this species belongs to 1980,
1203 thereafter it apparently disappeared from the island.

1204

1205 **1900-2005**

1206 Elba: 1908, 1909, 1916, 1917, 1921, 1963, 1976, 1980 (Biermann & Hesch, 1982 , Balletto et
1207 al., 2007).

1208

1209 *Not recorded on Elba in the last 10 years.*

1210

1211 We didn't collect any specimen of this species in Tuscan islands. Over the study area *L.*
1212 *sinapis* showed a low diversification among haplotypes (maximum p-distance 0.3%), but
1213 their distribution revealed a good population diversification (Dst 0.081 and Gst 0.501) since
1214 the haplotypes occurring on Sardinia and Corsica differ from those detected on the Tuscan
1215 mainland.

1216
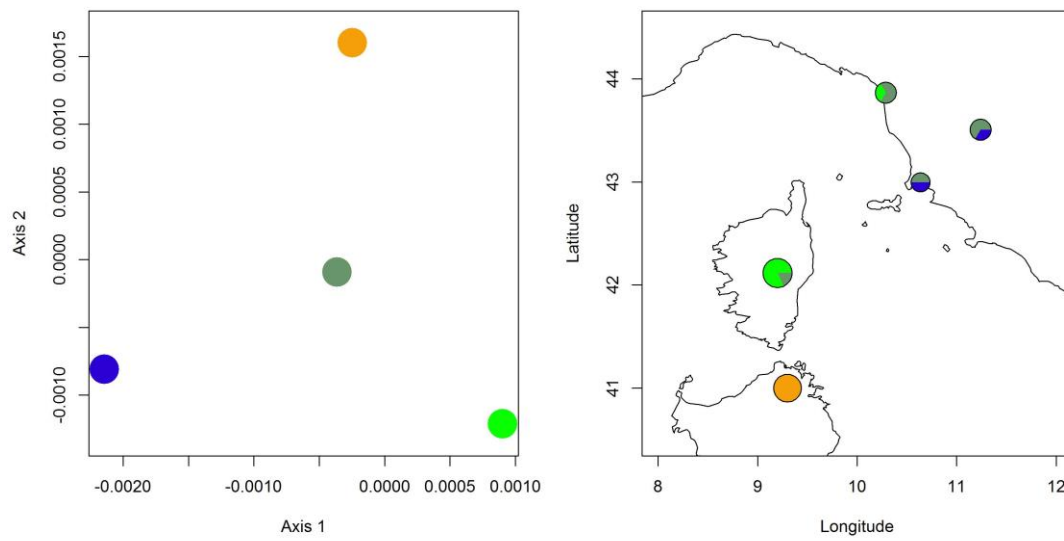
1217

1218

1219 Figure S15. PCoA projection (left) of genetic distances among specimens (dots) in the
1220 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1221 map (right). The first two PCoA axes explained 100% of variance.

1222   *Lycaena phlaeas* (Linnaeus, 1761)

1223

1224   **1900-2005**

1225   Elba: 1908, 1916, 1921, 1963, 1976 (Biermann & Hesch, 1982 , Balletto et al., 2007), 1998
1226   (Biermann, 1999), 2001-2004 (authors).

1227   Capraia: 1979 (Biermann & Hesch, 1982).

1228   Giglio: 1908 (Rocci & Turati, 1925), 1980 (Biermann & Hesch, 1982).

1229   Pianosa: 1998 (Dapporto et al. 1999).

1230   **2006-2015**

1231   Elba:, 2008-2013 (authors).

1232   Giglio: 2007, 2008, 2010, 2014 (authors).

1233   Montecristo: 2014 (authors).

1234   Pianosa: 2011 (authors).

1235

1236   *Not recorded on Capraia in the last 10 years.*

1237

1238   This species showed a low diversification among haplotypes (maximum p-distance 0.5%), but
1239   their distribution revealed a high population diversification (Dst 0.098 and Gst 0.762) for the
1240   occurrence on Corsica and Montecristo of haplotypes not detected on the Tuscan mainland.
1241   These haplotypes were not found elsewhere in Europe resulting in a uniqueness of 0.051.

1242

1243

1244

Figure S16. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 99.60% of variance.

1248

1249

1250 *Favonius quercus* (Linnaeus, 1758)

1251

1252 **1900-2005**

1253 Elba: 1908, 1916, 1921, 1963, 1980 (Biermann & Hesch, 1982 and Balletto et al. 2007), 2003
1254 (authors).

1255 **2006-2015**

1256 Elba: 2008, 2010, 2012, 2015 (authors).

1257

1258 This species showed a low diversification among haplotypes (maximum p-distance 0.5%), but
1259 their distribution revealed a high population diversification (Dst 0.053 and Gst 0.393). The
1260 haplotype detected on Elba was not found elsewhere in Europe resulting in a uniqueness of
1261 0.051.

1262
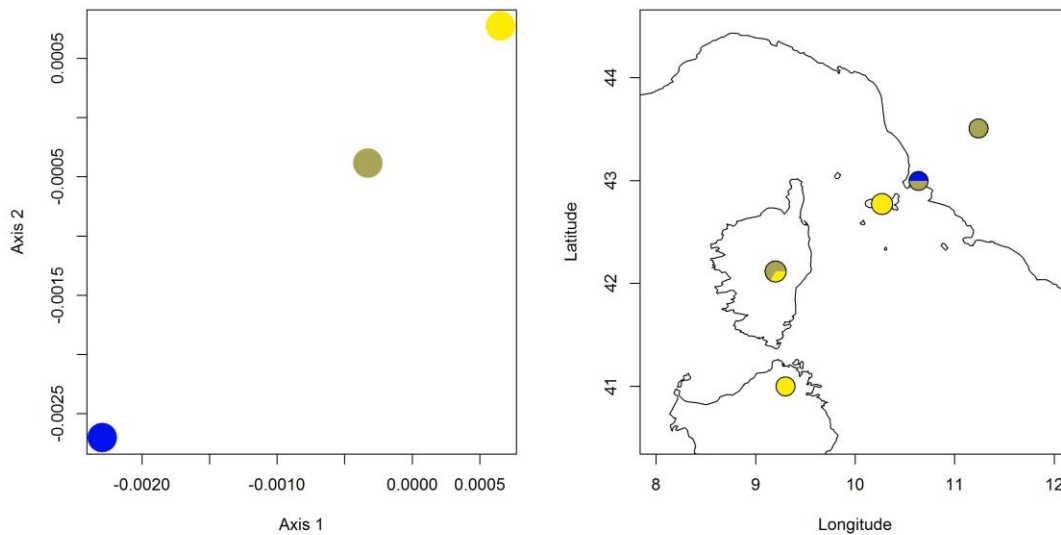
1263

1264

1265 Figure S17. PCoA projection (left) of genetic distances among specimens (dots) in the
1266 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1267 map (right). The first two PCoA axes explained 100% of variance.

1268

1269

1270 *Callophrys rubi* (Linnaeus, 1758)

1271

1272 **1900-2005**

1273 Elba: 1998 (Biermann, 1999).

1274 **2006-2015**

1275 Elba: 2008, 2009, 2011, 2013, 2014 (authors).

1276 Giglio: 2010 (authors).

1277

1278 This species showed some diversification among haplotypes (maximum p-distance 0.9%),
1279 and their distribution revealed a high population diversification (Dst 0.279 and Gst 0.846) for
1280 the occurrence on all the studied islands of a group of haplotypes not detected on the
1281 Tuscan mainland. However, since these haplotypes also occur in western Europe (Iberia)
1282 this species did not score island uniqueness.

1283
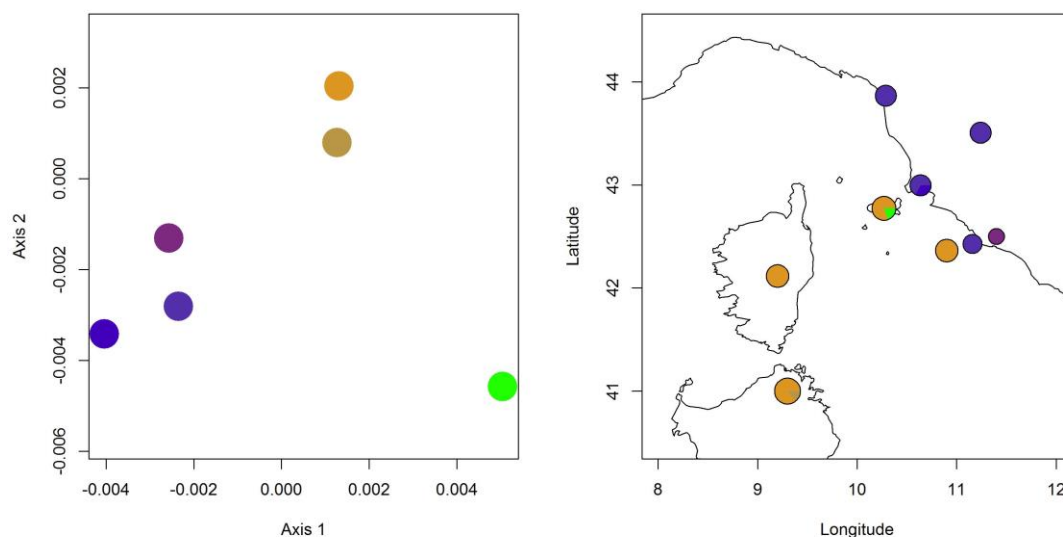
1284

1285

1286 Figure S18. PCoA projection (left) of genetic distances among specimens (dots) in the
1287 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1288 map (right). The first two PCoA axes explained 99.19% of variance.

1289

1290 *Satyrium ilicis* (Esper, 1779)

1291

1292 **1900-2005**

1293 Elba: 1908, 1916, 1921, 1963, 1968, 1978, 1980 (Biermann & Hesch, 1982; Balletto et al.
1294 2007).

1295 **2006-2015**

1296 Elba: 2008, 2010, 2015 (authors).

1297

1298 This species showed a single haplotype in the 11 specimens we analyzed from Tuscany
1299 mainland, Elba and Argentario.

1300

1301

1302

1303 *Lampides boeticus* (Linnaeus, 1767)

1304

1305 **1900-2005**

1306 Elba: 1908, 1916, 1921, 1963, 1968 (Biermann & Hesch, 1982, Balletto et al. 2007), 1998
1307 (Biermann, 1999), 2000-2003 (authors).

1308 Gorgona: 1999-2002 (Dapporto, 2004).

1309 Capraia: 1968-1970, 1996 (revised by Balletto et al. 2007).

1310 Giglio: 1980 (Biermann & Hesch, 1982), 2004 (authors).

1311 **2006-2015**

1312 Elba: 2008-2010, 2012 (authors).

1313 Capraia: 2007, 2008, 2012 (authors).

1314 Montecristo: 2012 (authors).

1315 Giglio: 2007, 2008, 2010, 2013, 2014 (authors).

1316 Gorgona: 2015 (authors).

1317 Pianosa: 2014 (authors).

1318

1319 This species showed a low diversification among haplotypes (maximum p-distance 0.6%),
1320 and their distribution was not spatially structured resulting in low population diversification
1321 (Dst 0.005 and Gst 0.045). The detection on Capraia of a haplotype not found on the
1322 mainland resulted in a uniqueness value of 0.038.

1323
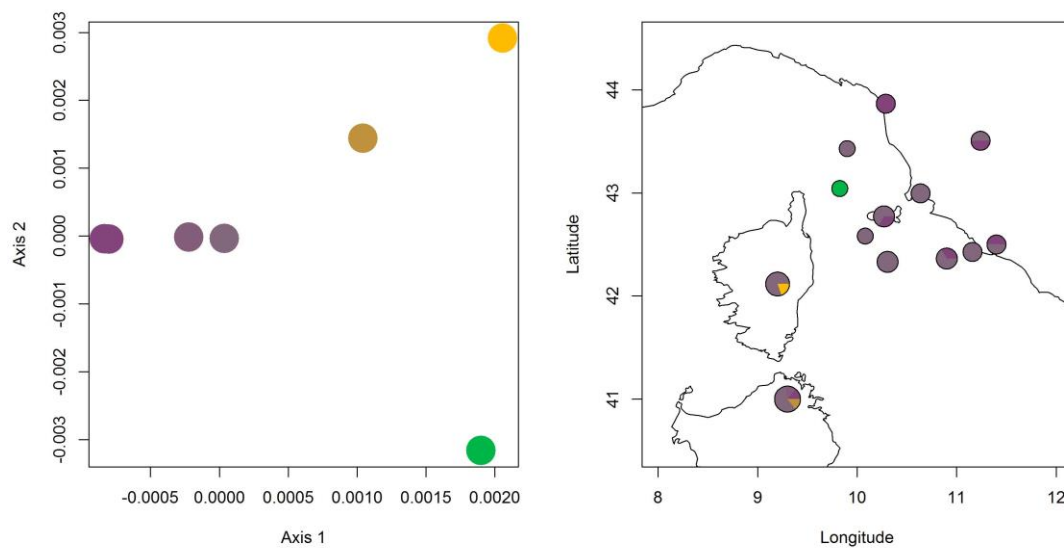
1324

1325

Figure S19. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 55.88% of variance.

1329

1330

1331 *Leptotes pirithous* (Linnaeus, 1767)

1332

1333 **1900-2005**

1334 Elba: 1908, 1916, 1921, 1951, 1963, 1980 (Biermann & Hesch, 1982, Balletto et al. 2007),
1335 2000, 2002, 2004 (authors).

1336 Capraia: 1914 (Razzauti 1917); 1979 (Biermann & Hesch, 1982), 2000 (authors).

1337 Gorgona: 1999-2002 (Dapporto, 2004).

1338 Giglio:

1339 **2006-2015**

1340 Elba: 2008, 2010, 2013, 2014 (authors).

1341 Giglio: 2010, 2012, 2013, 2014 (authors).

1342 Capraia: 2006, 2010 (authors).

1343 Montecristo: 2012 (authors).

1344 Giannutri: 2014 (authors).

1345

1346 This species showed a low diversification among haplotypes (maximum p-distance 0.6%),
1347 and their spatially structure was low (Dst 0.048 and Gst 0.298). All the haplotypes found on
1348 Tuscan islands also occurred on mainland.

1349
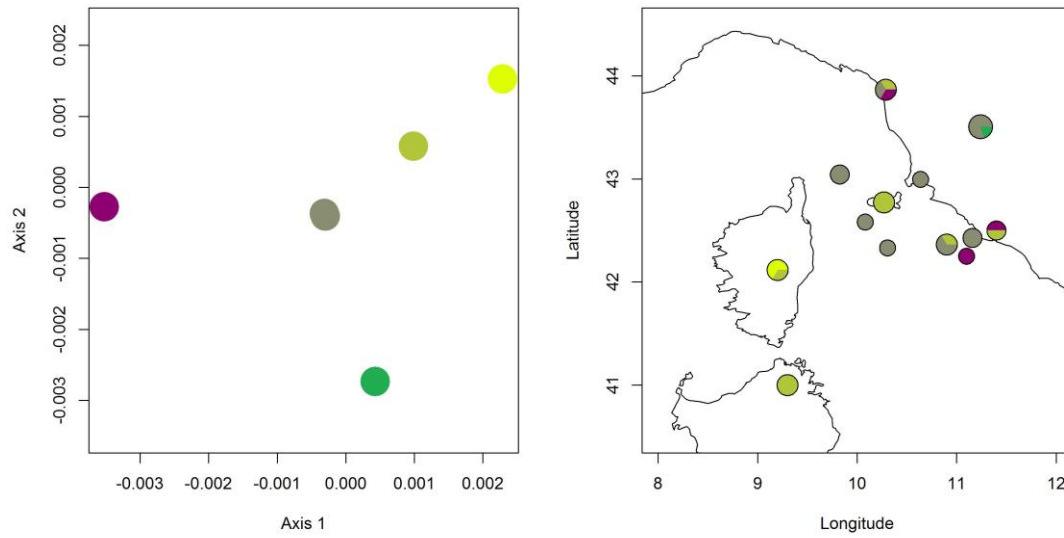
1350

1351

Figure S20. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 99.79% of variance.

1355

1356

1357 *Celastrina argiolus* (Linnaeus, 1758)

1358

1359 **1900-2005**

1360 Elba: 1908, 1916, 1963, 1980 (Biermann & Hesch, 1982, Balletto et al. 2007), 1998
1361 (Biermann, 1999), 2002, 2003 (authors).

1362 Capraia: 1968-1970, 1978-1980, 1996 (revised by Biermann & Hesch, 1982; Balletto et al.
1363 2007), 2001, 2002 (authors).

1364 Giglio: 1908, 1979 (revised by Biermann & Hesch, 1982; Balletto et al. 2007).

1365 Gorgona: 1999-2002 (Dapporto, 2004).

1366 **2006-2015**

1367 Elba: 2008-2010, 2013-2015 (authors).

1368 Capraia: 2006-2008, 2010-2014 (authors).

1369 Giglio: 2007, 2008, 2012, 2013, 2014 (authors).

1370 Gorgona: 2015 (authors).

1371 Montecristo: 2014 (authors).

1372 Pianosa: 2011 (authors).

1373

1374 This species showed a low diversification among haplotypes (maximum p-distance 0.3%),
1375 and their distribution was not spatially structured resulting in low population diversification
1376 (Dst 0.010 and Gst 0.114). The detection on Elba and Giglio of a haplotype not found on the
1377 mainland resulted in a uniqueness value of 0.076.

1378
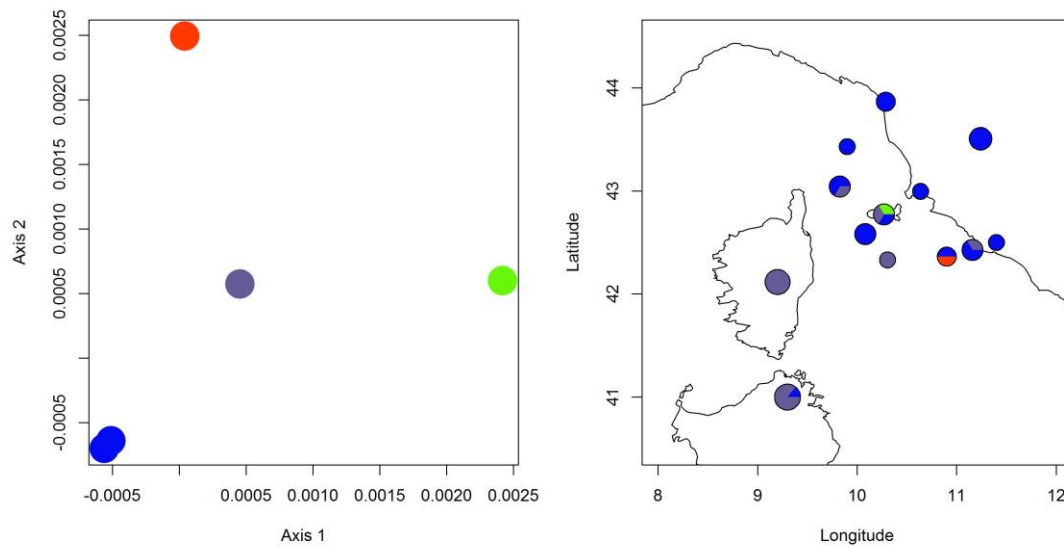
Figure S21. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 99.98% of variance.

1386 *Glaucopsyche alexis* (Poda, 1761)

1387

1388 **1900-2005**

1389 Elba: 1916 (revised by Biermann & Hesch, 1982).

1390 **2006-2015**

1391 Elba: 2008, 2009, 2013, 2014 (authors).

1392

1393 This species showed a high diversification among haplotypes (maximum p-distance 2.0%),
1394 but their distribution was not spatially structured resulting in no population diversification
1395 (Dst and Gst were 0). The haplotype found on Elba also occurs on the European mainland.

1396
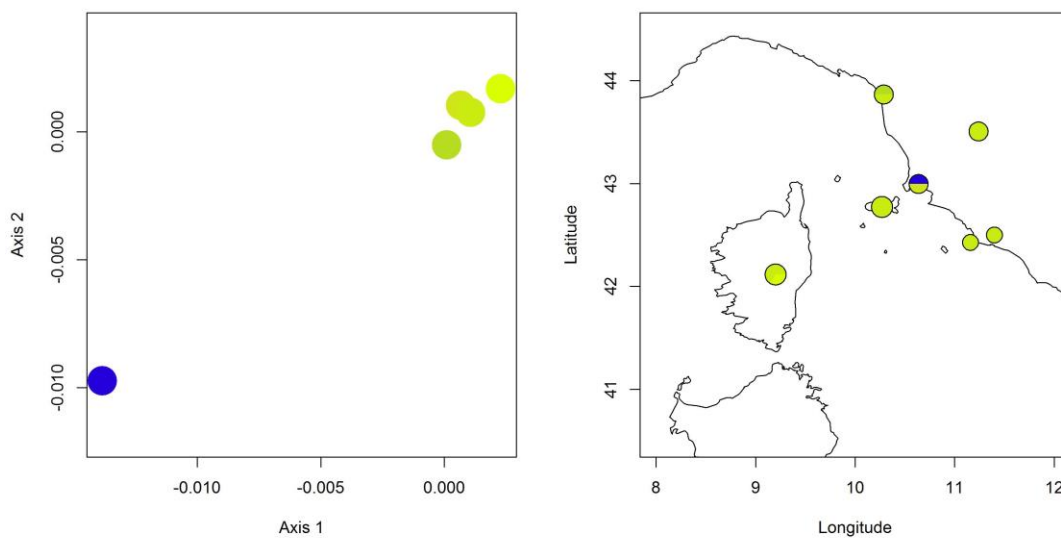
1397

1398

1399 Figure S22.   PCoA projection (left) of genetic distances among specimens (dots) in the
1400 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1401 map (right). The first two PCoA axes explained 100% of variance.

1402

1403

1404 *Plebejus idas/bellieri*

1405

1406 The taxonomy of this group is still debated. The Sardo-Corsican populations as referred as *P.*
1407 *bellieri*, the Elba population is referred by some authors as *P. villai* or *P. bellieri villai* and the
1408 Tuscan populations as *P. idas* or *P. abetonica*). According to COI there clear distinction
1409 between insular and mainland populations populations. For this reason, they have been
1410 analyzed together.

1411

1412 **1900-2005**

1413 Elba: 1958, 1963, 1973, 1976, 1978, 1979, 1980, 1993; 1998; 2002 (Biermann & Hesch, 1982,
1414 Balletto et al. 2007).

1415 **2006-2015**

1416 Elba: 2008, 2010, 2013 (authors).

1417

1418 This species showed some diversification among haplotypes (maximum p-distance 0.8%),
1419 and their distribution showed a good spatial structure resulting in Dst equal to 0.134 and Gst
1420 equal to 0.616. The detection on Elba of a haplotype not found on the mainland resulted in a
1421 uniqueness value of 0.051.

1422
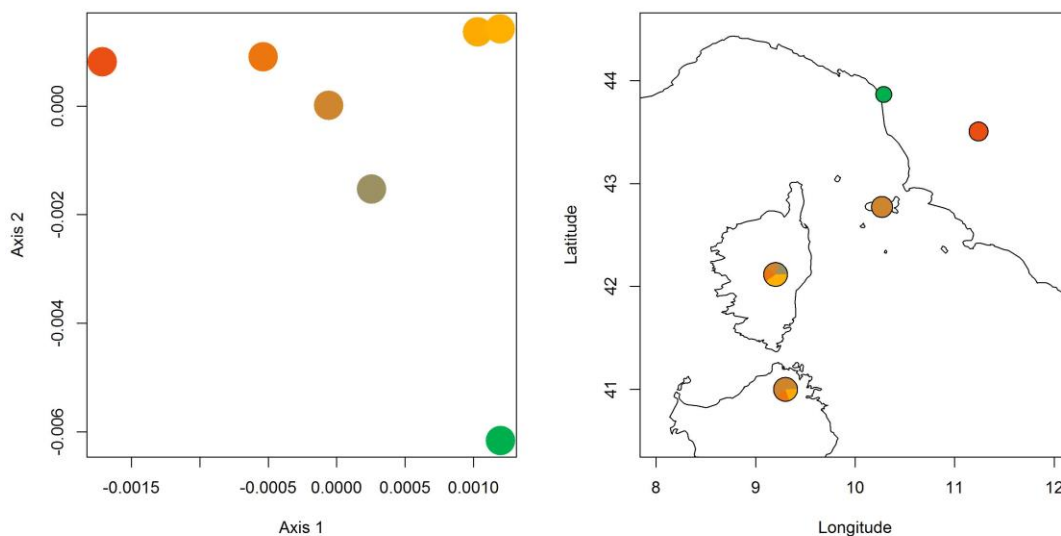
1423

1424

1425 Figure S23. PCoA projection (left) of genetic distances among specimens (dots) in the
1426 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1427 map (right). The first two PCoA axes explained 82.89% of variance.

1428

1429

1430 *Aricia agestis/cramera*

1431

1432 In Sardinia, *A. agestis* is replaced by the South-Western taxon *A. cramera*. The two taxa show
1433 a rather low COI divergence (around 2.4% depending on different haplotypes) (Sañudo-
1434 Restrepo et al., 2013). For this reason, *A. agestis* from Tuscany, Tuscan islands and Corsica
1435 and *A. cramera* from Sardinia have been used together in the analyses.

1436

1437 **1900-2005**

1438 Elba: 1908, 1916, 1920, 1921, 1951, 1957, 1963, 1968, 1976, 1980 (Biermann & Hesch, 1982;
1439 Balletto et al. 2007), 2000, 2002, 2003 (authors).

1440 Giglio: 1908 (Rocci and Turati, 1925), 1979 (Biermann & Hesch, 1982).

1441 Pianosa: 1998 (Dapporto et al. 1999).

1442 **2006-2015**

1443 Elba: 2008-2010, 2012-2015 (authors).

1444 Capraia: 2013 (authors).

1445

1446 *Not recorded on Giglio in the last 10 years.*

1447

1448 As reported above, this species showed a high diversification among two taxa (maximum p-
1449 distance 3.2%), and their distribution revealed a high population diversification (Dst 0.984
1450 and Gst 0.738) for the occurrence on all the studied islands of a group of haplotypes not
1451 detected on the Tuscan mainland. Some haplotypes were not recorded on the mainland and
1452 this species scored a uniqueness of 0.094.

1453
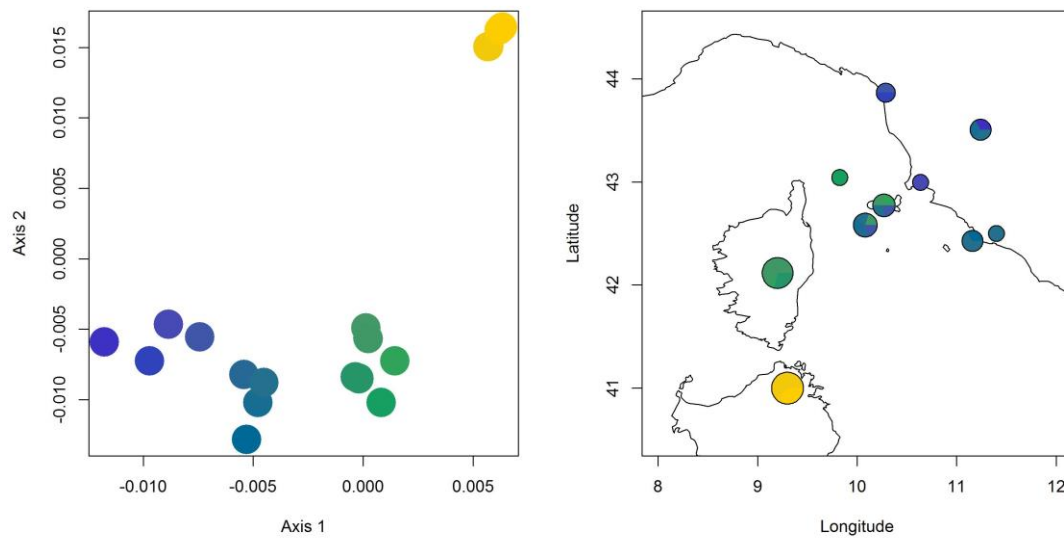
1454

1455

Figure S24. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 96.73

% of variance.

1460

1461

1462   *Polyommatus icarus* (Rottemburg, 1775)

1463

1464   It has been recently found that the populations from Sardinia belong to *P. celina*, a S-W
1465   Mediterranean taxon morphologically almost identical to *P. icarus*. The COI divergence is
1466   high (6%) and, for this reason, *P. celina* has been removed from the analysis of *P. icarus*.

1467

1468   **1900-2005**

1469   Elba: 1908, 1916, 1921, 1963, 1968, 1978, 1980 (Biermann & Hesch, 1982; Balletto et al.
1470   2007), 2000, 2002, 2003 (authors).

1471   Capraia: 1979 (Biermann & Hesch, 1982).

1472   Giglio: 1908, 1979 (Biermann & Hesch, 1982; Balletto et al. 2007).

1473   Pianosa: 1998 (Dapporto et al. 1999).

1474   **2006-2015**

1475   Elba: 2008-2010, 2012-2015 (authors).

1476   Capraia: 2013, 2014 (authors).
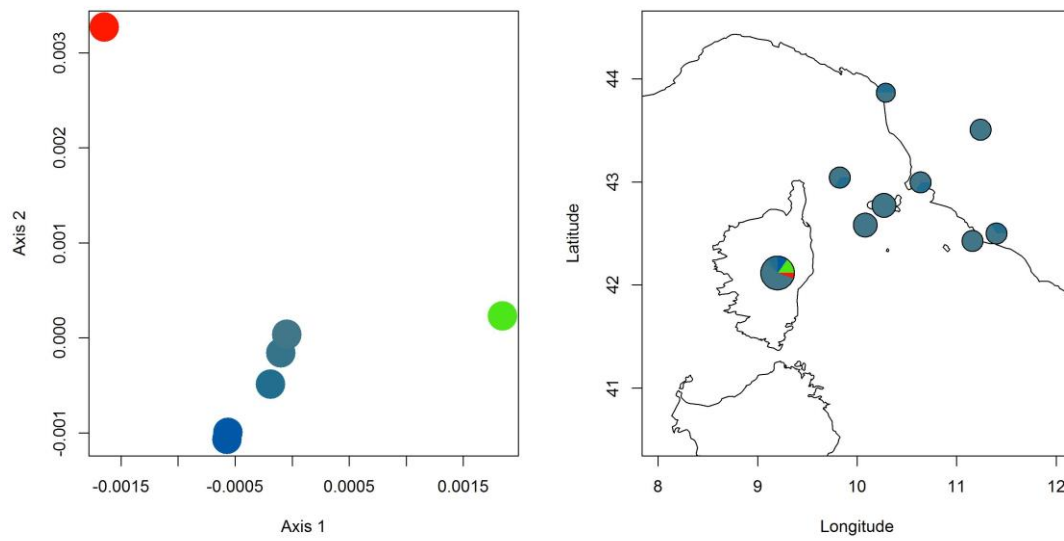
1477   Pianosa: 2011 (authors).

1478

1479   *Not recorded on Giglio in the last 10 years.*

1480

1481   This species showed a low diversification among haplotypes (maximum p-distance 0.5%) and
1482   in a rather low population diversification (Dst 0.018 and Gst 0.216). The occurrence on
1483   Capraia of a haplotype not found on the mainland resulted in an island uniqueness of 0.076.

1484

1485

1486

1487 Figure S25. PCoA projection (left) of genetic distances among specimens (dots) in the
1488 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1489 map (right). The first two PCoA axes explained 46.86% of variance.

1490

1491   *Argynnis paphia* (Linnaeus, 1758)

1492

1493   **1900-2005**

1494   Elba: 1908, 1916, 1921 (Biermann & Hesch, 1982; Balletto et al. 2007).

1495

1496   *Not recorded on Elba in the last 10 years.*

1497

1498   We didn't collect any specimen of this species in Tuscan islands. Over the study area
1499   (Tuscany, Corsica and Sardinia) this species showed a single haplotype.

1500

1501   *Argynnis pandora* (Denis & Schiffermüller, 1775)

1502

1503   **1900-2005**

1504   Elba: 1908, 1909, 1916, 1921, 1961, 1963, 1978, 1980 (Biermann & Hesch, 1982; Balletto et
1505   al. 2007), 2002 (authors).

1506   Giglio: 1908 (Rocci & Turati, 1925), 1979 (Biermann & Hesch, 1982).

1507   **2006-2015**

1508   Elba: 2008, 2009, 2010, 2012, 2013, 2015 (authors).

1509   Giglio: 2008, 2010, 2012, 2013, 2014 (authors).

1510

1511   This species showed a high diversification among haplotypes (maximum p-distance 2.6%),
1512   but their distribution was not highly spatially structured resulting a low population Gst
1513   (0.171) and in a medium Dst (0.150). One haplotype detected on Giglio and Elba was not
1514   found on the mainland resulting in a uniqueness value of 0.076.

1515
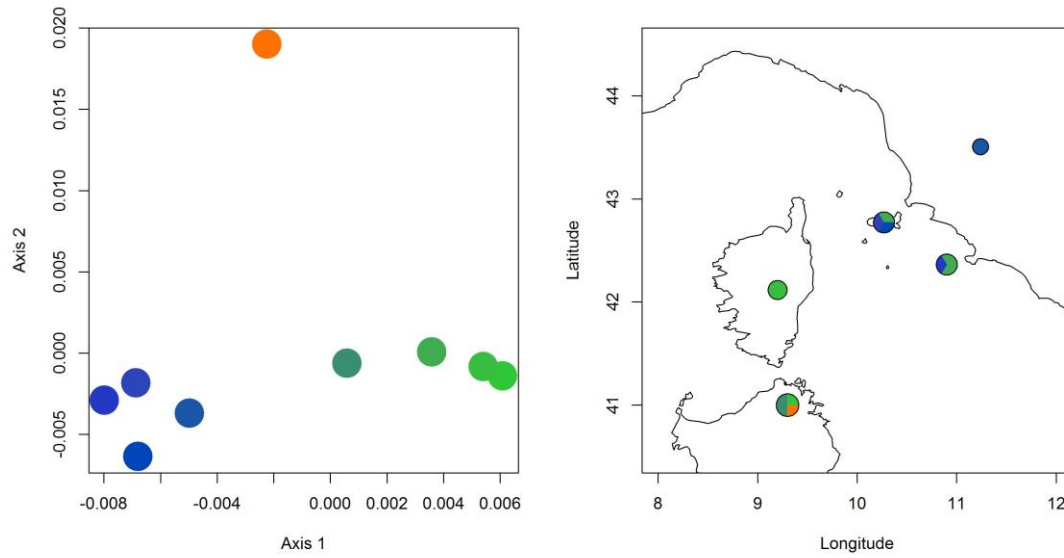
1516

1517

1518 Figure S26. PCoA projection (left) of genetic distances among specimens (dots) in the
1519 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1520 map (right). The first two PCoA axes explained 98.05 % of variance.

1521

1522

1523   *Issoria lathonia* (Linnaeus, 1758)

1524

1525   **1900-2005**

1526   Elba: 1908, 1916, 1921, 1963, 1978, 1980 (Biermann & Hesch, 1982; Balletto et al. 2007),
1527   1998 (Biermann, 1999).

1528   Giglio: 1908 (Rocci & Turati, 1925), 1979 (Biermann & Hesch, 1982).
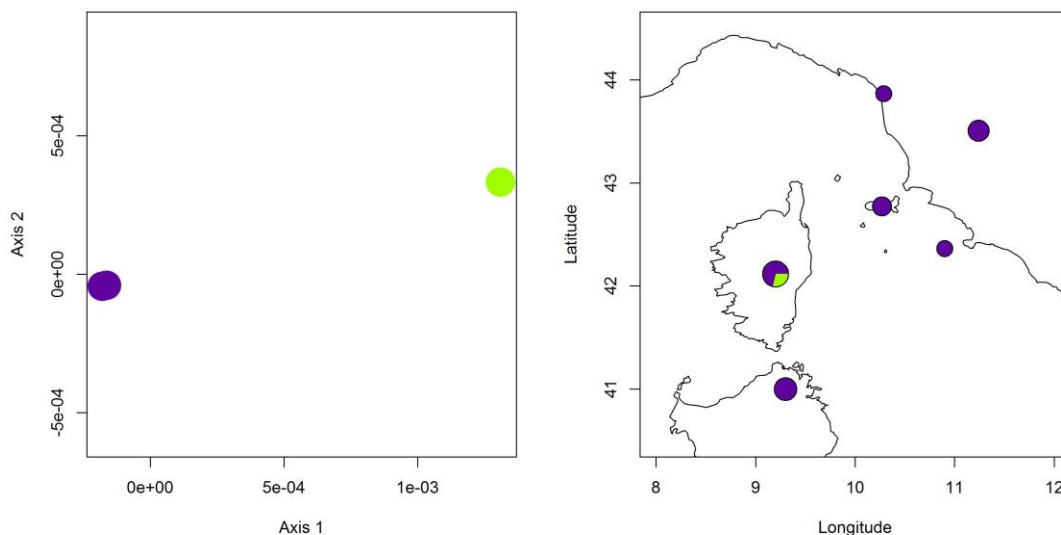
1529   **2006-2015**

1530   Elba: 2008-2010, 2012-2015 (authors).

1531   Giglio: 2014 (authors).

1532

1533   This species showed only three haplotypes only slightly differentiated for a single mutation.
1534   One of them has been only found in Corsica resulting in some Gst (0.431) in a low Dst (0.014)
1535   and in a zero value for island uniqueness.

1536

1537

1538

1539   Figure S27. PCoA projection (left) of genetic distances among specimens (dots) in the
1540   bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1541   map (right). The first two PCoA axes explained 100% of variance.

1542

1543

1544   *Aglais urticae/ichnusa*

1545

1546   In Sardinia and Corsica, *A. urticae* is replaced by the island endemic *A. ichnusa*, showing a
1547   low COI divergence (around 1.2% in our sample) (see also Vandewoestijne et al., 2004). For
1548   this reason, the two species have been analyzed together. The two taxa differ for wing
1549   pattern and the specimen collected on Elba island by Roger Verity (still preserved in his
1550   collection) revealed a typical *A. urticae* wing pattern.

1551

1552   **1900-2005**
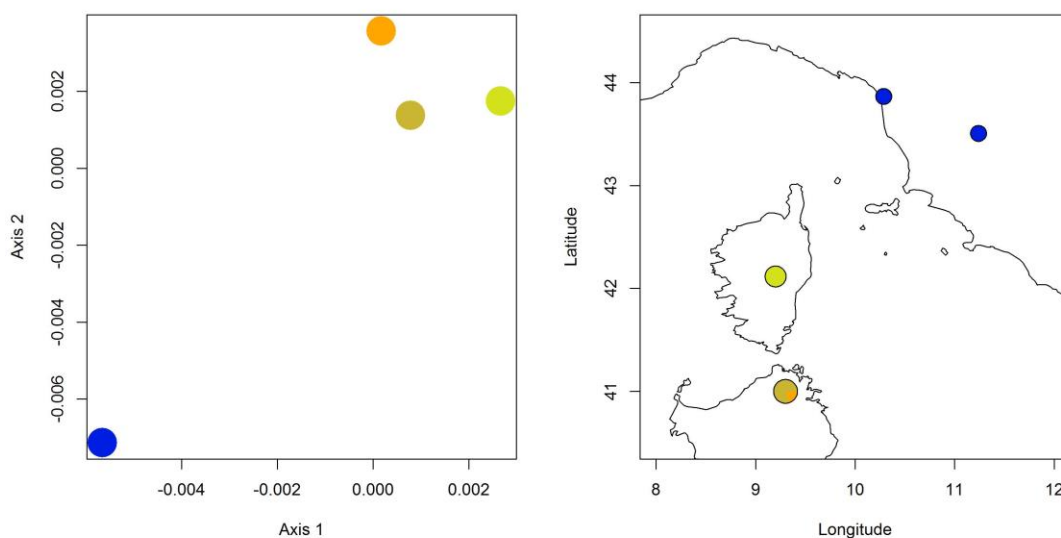
1553   Elba: 1908, 1916 (Biermann & Hesch, 1982).

1554

1555   *Not recorded on Elba in the last 10 years.*

1556

1557   According to the existence of two taxa, *A. urticae* and *A ichnusa* showed a high
1558   diversification between Tuscany and Corsica-Sardinia (maximum divergence 1.2%, Gst
1559   (0.934) and Dst (0.449).

1560

1561

1562

1563   Figure S28. PCoA projection (left) of genetic distances among specimens (dots) in the
1564   bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1565   map (right). The first two PCoA axes explained 100% of variance.

1566

1567

1568   *Nymphalis polychloros* (Linnaeus, 1758)

1569

1570   **1900-2005**

1571   Elba: 1908, 1916 (Biermann & Hesch, 1982; Balletto et al. 2007)

1572

1573   In the study area this species showed a series of haplotypes with a low level of diversification
1574   (maximum p-distance 0.3%). However, they revealed some spatial structured resulting in a
1575   rather low value of Dst due to the low diversification (0.043) but in a medium values of Gst
1576   (0.415).

1577

1578   *Not recorded on Elba in the last 10 years.*

1579
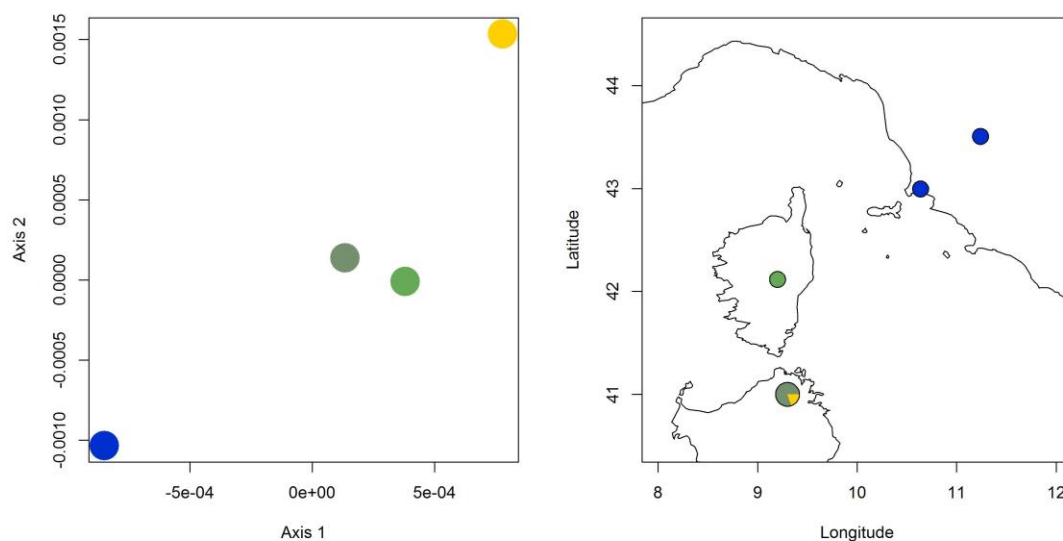
1580

1581

1582   Figure S29. PCoA projection (left) of genetic distances among specimens (dots) in the
1583   bidimensional RGB color space. Individual colours have been subsequently plotted on the
1584   map (right). The first two PCoA axes explained 100% of variance.

1585

1586

1587   *Vanessa atalanta* (Linnaeus, 1758)

1588

1589   **1900-2005**

1590   Elba: 1908, 1916, 1921, 1956, 1963, 1978, 1980, 2007 (Biermann & Hesch, 1982, Balletto et
1591   al. 2007), 1998 (Biermann, 1999).

1592   Capraia: 1915 (Razzauti, 1917), 1978 (Biermann & Hesch, 1982), 2002 (authors).

1593   Giglio: 1908, 1979 (Biermann & Hesch, 1982; Balletto et al. 2007), 2003 (authors).

1594   Montecristo: 1983 (Raineri, 1986), 2003 (authors).

1595   Gorgona: 1999-2002 (Dapporto 2004).

1596   Pianosa: 1998 (Dapporto et al., 1999).

1597   **2006-2015**

1598   Elba: 2008-2015 (authors).

1599   Capraia: 2006-2008, 2010, 2012-2014 (authors).

1600   Giglio: 2007, 2008, 2010, 2012, 2013 (authors).

1601   Montecristo: 2012, 2014 (authors).

1602

1603   This species showed a low diversification among haplotypes (maximum p-distance 0.5%),
1604   and their distribution was not spatially structured resulting in low population diversification
1605   (Dst 0.009 and Gst 0.126). The detection on Capraia and Elba of two haplotypes not found
1606   on the mainland resulted in a uniqueness value of 0.051.

1607
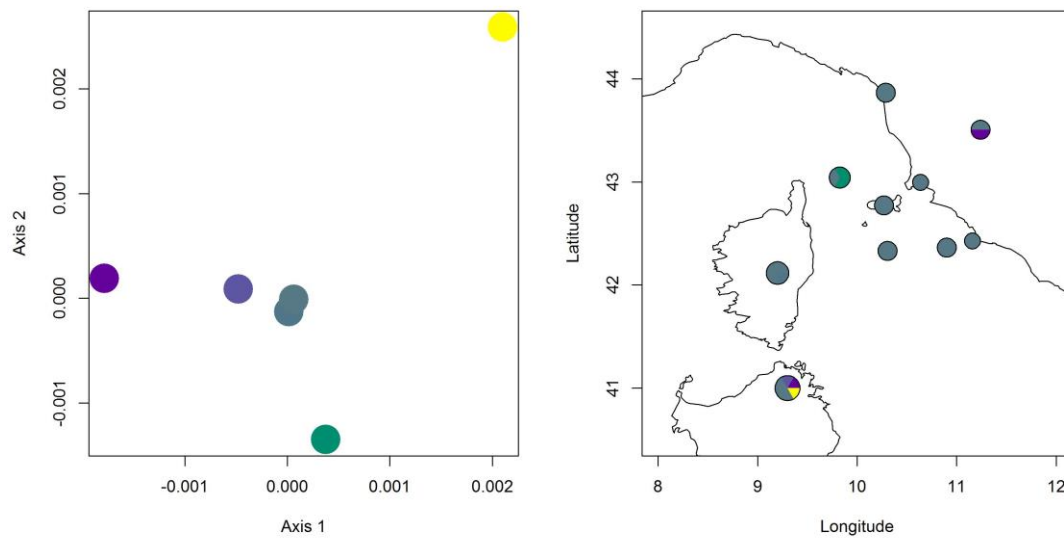
1608

1609

Figure S30. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 82.39% of variance.

1613

1614

1615   *Vanessa cardui* (Linnaeus, 1758)

1616

1617   **1900-2005**

1618   Elba: 1908, 1916, 1921, 1963, 1978, 1980 (Biermann & Hesch, 1982, Balletto et al. 2007),
1619   1998 (Biermann, 1999), 2000-2003 (authors).

1620   Capraia: 1915 (Razzauti, 1917), 1968-1970 (Gross, 1970), 1978 (Biermann & Hesch, 1982),
1621   2001, 2003 (authors).

1622   Giglio: 1908 (Rocci & Turati, 1925), 1979 (Biermann & Hesch, 1982).

1623   Pianosa: 1998 (Dapporto et al., 1999).

1624   Montecristo: 1979 (Fanfani & Groppali, 1979).

1625   **2006-2015**

1626   Elba: 2006, 2008-2010, 2012-2015 (authors).

1627   Capraia: 2006-2008, 2012, 2014 (authors).

1628   Giglio: 2007, 2013 (authors).

1629   Montecristo: 2012, 2014 (authors).

1630

1631   This species showed a low diversification among haplotypes (maximum p-distance 0.8%),
1632   and their distribution was not spatially structured resulting in very low population
1633   diversification (Dst 0.007 and Gst 0.032). The detection on Gorgona and Giannutri of a
1634   haplotype not found on the mainland resulted in a uniqueness value of 0.038

1635
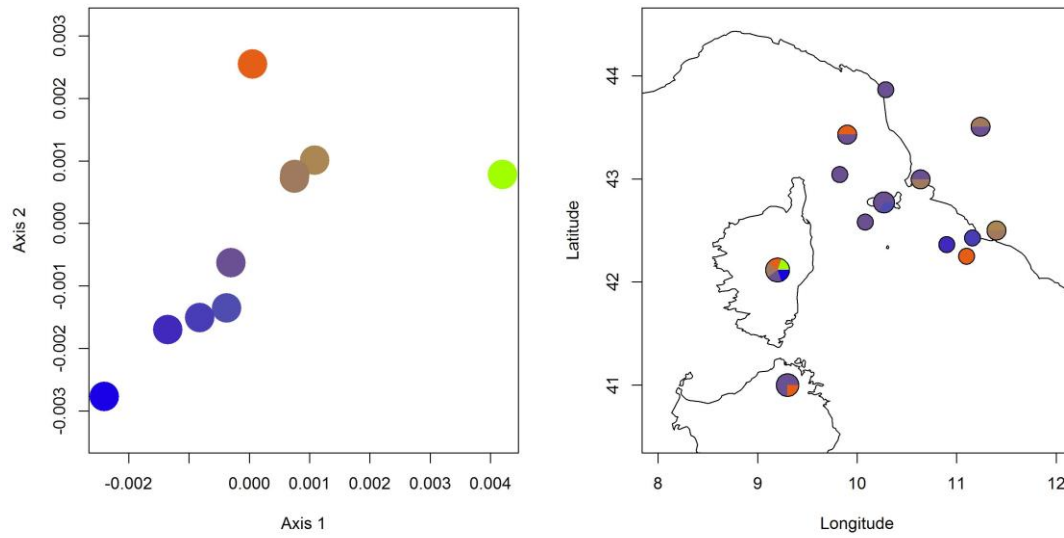
1636

1637

1638 Figure S31. PCoA projection (left) of genetic distances among specimens (dots) in the
1639 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1640 map (right). The first two PCoA axes explained 76.06% of variance.

1641

1642

1643 *Melitaea didyma* (Esper, 1778)

1644

1645 **1900-2005**

1646 Elba: 1908, 1916, 1917, 1921, 1924, 1956, 1957, 1961, 1963, 1968, 1978, 1980 (Biermann &
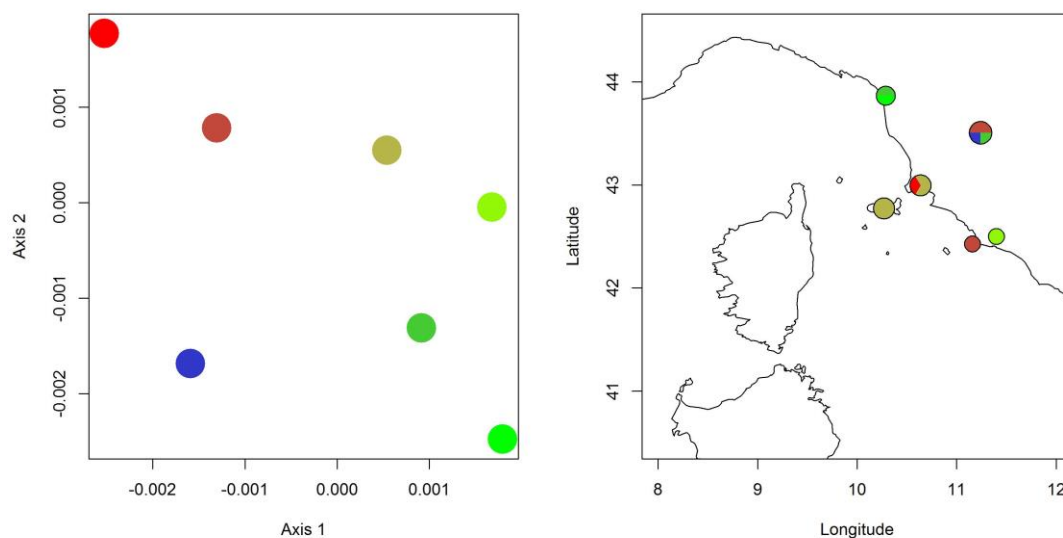1647 Hesch, 1982, Balletto et al. 2007).

1648 **2006-2015**

1649 Elba: 2012, 2015 (authors).

1650

1651 This species showed a low diversification among haplotypes (maximum p-distance 0.6%) but
1652 their distribution revealed some spatial structure (Dst 0.070 and Gst 0.344). The haplotype
1653 from Elba has been also found on the mainland.

1654

1655

1656

1657 Figure S32. PCoA projection (left) of genetic distances among specimens (dots) in the
1658 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1659 map (right). The first two PCoA axes explained 93.13% of variance.

1660

1661

1662 *Melitaea nevadensis* (Oberthür, 1904)

1663

1664 **1900-2005**

1665 Elba: 1908, 1916, 1917, 1921, 1978 (Biermann & Hesch, 1982; Balletto et al. 2007), 2002
1666 (authors).

1667 **2006-2015**

1668 Elba: 2015 (authors).

1669

1670 This species revealed a high intra-specific diversification in the study area (2.1%). The
1671 existence of two closely related haplotypes from Elba not found elsewhere resulted in high
1672 population differentiation (Dst 0.492, Gst 0.454 and Uni 0.989). The haplotypes found on
1673 Elba resulted in the highest uniqueness measured in this study.

1674
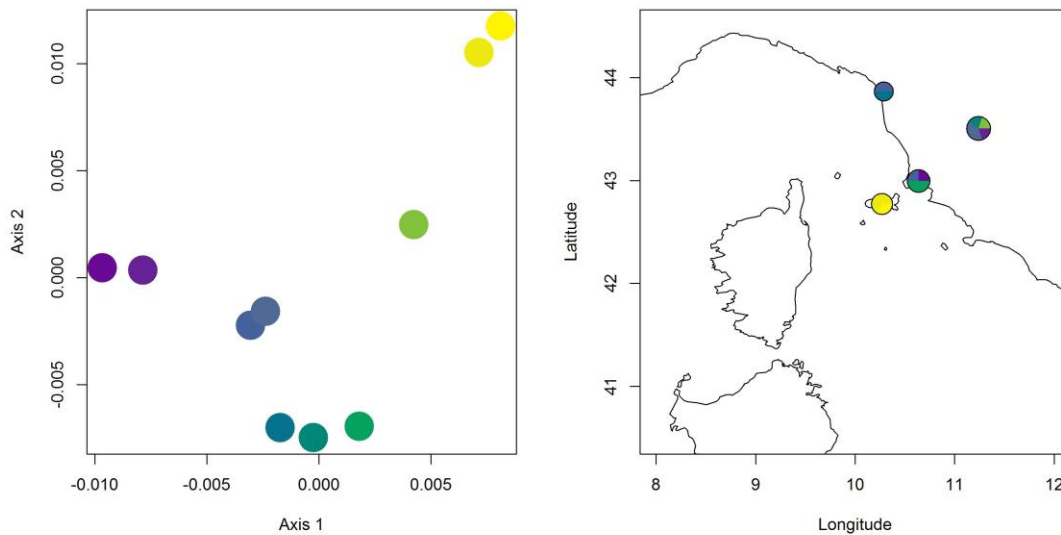
1675

1676

1677 Figure S33. PCoA projection (left) of genetic distances among specimens (dots) in the
1678 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1679 map (right). The first two PCoA axes explained 91.39% of variance.

1680

1681

1682   *Limenitis reducta* Staudinger, 1901

1683

1684   **1900-2005**

1685   Elba: 1908, 1916, 1921, 1963, 1978, 1980 (Biermann & Hesch, 1982; Balletto et al. 2007).

1686   **2006-2015**

1687   Elba: 2006-2013 (authors).

1688

1689   This species showed a low diversification among haplotypes (maximum p-distance 0.3%) and
1690   their distribution showed a very low spatial structure (Dst 0.015 and Gst 0.185). The
1691   haplotypes from Elba have been also found on the mainland.

1692
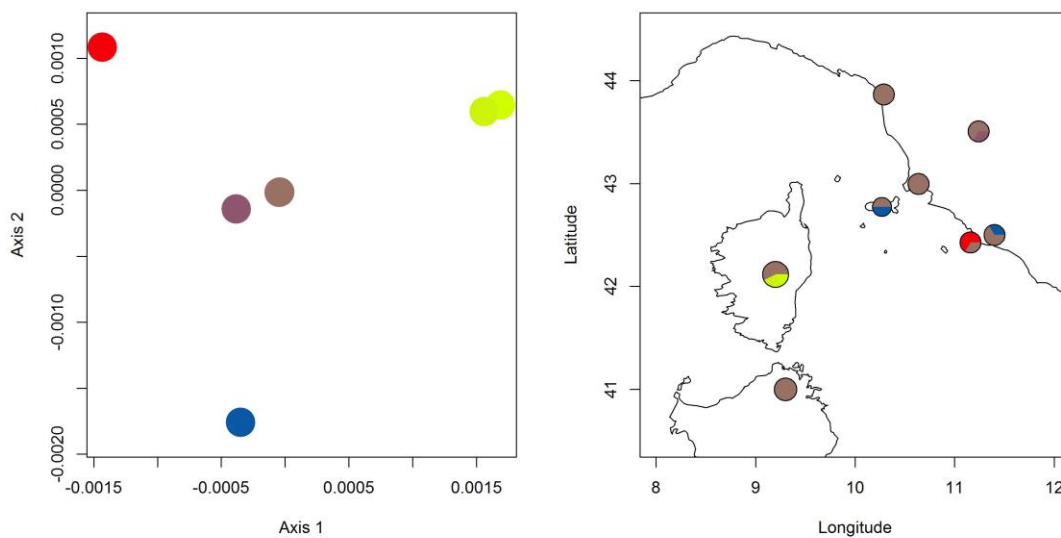
1693

1694

1695   Figure S34. PCoA projection (left) of genetic distances among specimens (dots) in the
1696   bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1697   map (right). The first two PCoA axes explained 79.48% of variance.

1698

1699

1700 *Charaxes jasius* (Linnaeus, 1767)

1701

1702 **1900-2005**

1703 Capraia: 1996 (Jutzeler & de Bros, 1997), 2000, 2001 (authors).

1704 Elba: 1908, 1916, 1920, 1921, 1963, 1980 (Biermann & Hesch, 1982; Balletto et al. 2007),
1705 2000 (authors)

1706 Giglio: 1908 (Rocci & Turati, 1925), , 1979 (Biermann & Hesch, 1982), 2004 (authors).

1707 Gorgona: 2004 (Dapporto & Cini, 2007) [without collection date]

1708 Pianosa: 2005 (Dapporto & Cini, 2007) [without collection date]

1709 **2006-2015**

1710 Elba: 2006-2009, 2011, 2013, 2014 (authors).

1711 Giglio: 2010, 2013 (authors).

1712 Capraia: 2007, 2010, 2014 (authors).

1713 Giannutri: 2014 (authors).

1714

1715 This species showed three haplotypes only differentiated for a few mutations and with a
1716 maximum diversification of 0.2%. One of the haplotypes has been only found on Sardinia
1717 resulting in negative Gst and Dst values which have been set to zero. The haplotype from
1718 Tuscan islands has been also found on the mainland.

1719

1720

1721

Figure S35. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 100% of variance.

1725

1726

1727 *Pararge aegeria* (Linnaeus, 1758)

1728

1729 **1900-2005**

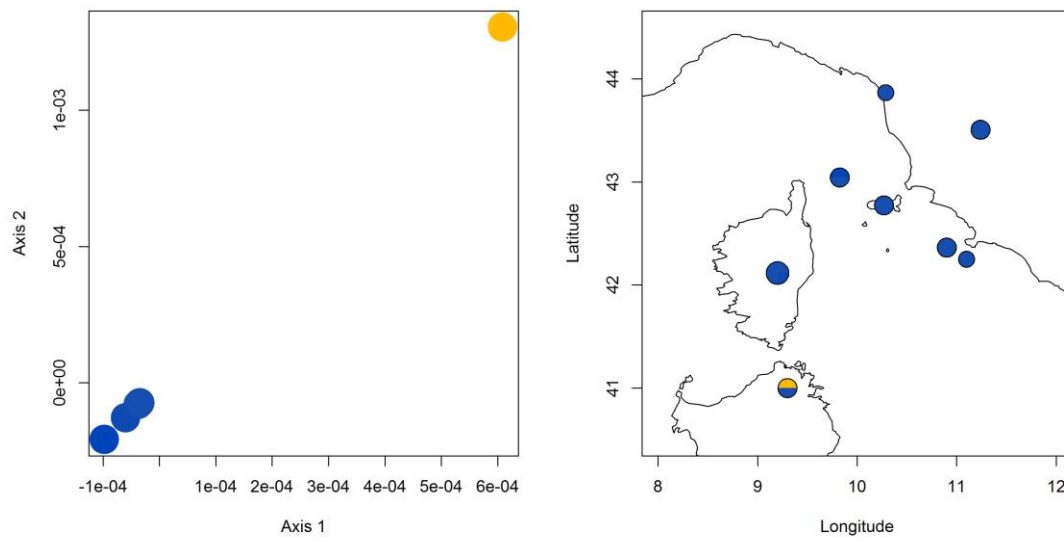1730 Elba: 1908, 1916, 1919, 1921, 1968, 1980 (Biermann & Hesch, 1982; Balletto et al. 2007),
1731 1998 (Biermann, 1999), 2002, 2003 (authors)

1732 Montecristo: 1979 (Fanfani & Groppali 1979).

1733 Giglio: 1908 (Rocci & Turati 1925), 1979 (Biermann & Hesch, 1982).

1734 **2006-2015**

1735 Elba: 2008-2015 (authors).

1736 Capraia: 2008, 2010, 2014 (authors).

1737 Giglio: 2007, 2010, 2014 (authors)

1738 Giannuutri: 2014 (authors)

1739 Pianosa: 2011 (authors).

1740 Montecristo: 2014 (authors).

1741 Gorgona: 2015 (authors).

1742

1743 It is well known that this species shows two diverging clades between North Africa and
1744 Europe (Weingartner et al. 2006; Vodă et al. 2016) (2.1% of maximum divergence in our
1745 dataset). We found that the North African lineage also occurs on Sardinia and a single
1746 specimen has been also found on Corsica. All the specimens from Tuscan islands revealed to
1747 belong to the European clade. According to this diversification the species revealed a high
1748 population differentiation (Dst 0.636, Gst 0.880); the occurrence on Capraia and Pianosa of a
1749 haplotype not found on the mainland resulted in a uniqueness value of 0.076.

1750

1751
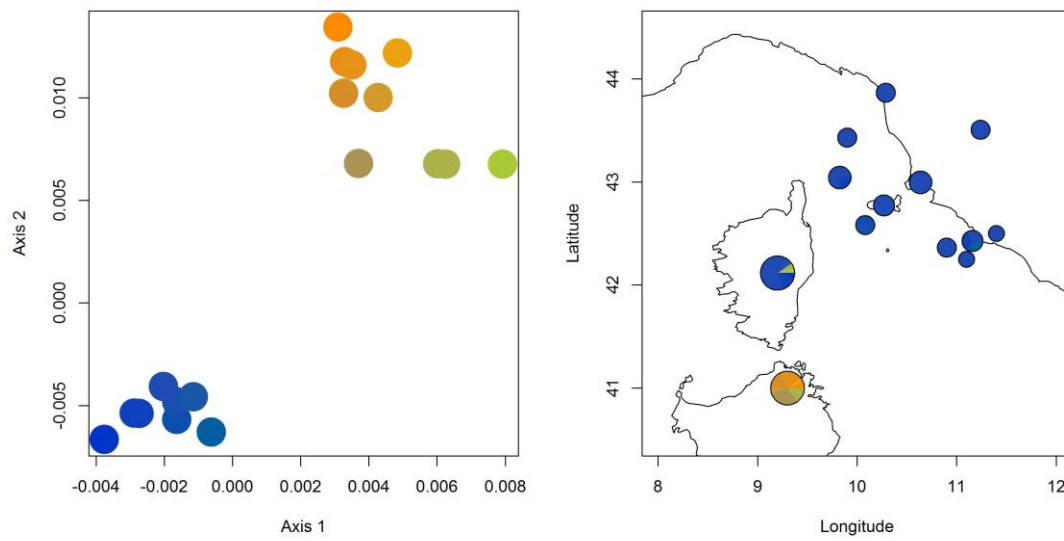
1752

1753

Figure S36.   PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 96.10% of variance.

1757

1758

1759 *Lasiommata megera/paramegaera*

1760

1761 *Lasiommata megera* is replaced on Sardinia, Corsica and Capraia by the insular endemic
1762 taxon *L. paramegaera*, differentiated in wing pattern, genitalia shape and composition of
1763 cuticular lipids (e.g. Kudrna 1977; Dapporto 2007, 2008). To our knowledge no COI sequence
1764 was available in literature for *L. paramegaera*. Based on our sequences the genetic distance
1765 in COI between *L. megera* and *L. paramegaera* is lower than 3% (1.5% of maximum
1766 divergence). For this reason we analysed the two taxa together.

1767

1768 **1900-2005**

1769 Elba: 1908, 1916, 1921, 1963, 1968, 1980 (Biermann & Hesch, 1982; Balletto et al. 2007),
1770 1998 (Biermann, 1999), 2000-2003 (authors)

1771 Montecristo: 1979 (Fanfani & Groppali 1979), 2001 (authors).

1772 Giglio: 1908 (Rocci & Turati 1925), 1979 (Biermann & Hesch, 1982), 2003, 2004 (authors).

1773 Gorgona: 1999-2002 (Dapporto 2004).

1774 Pianosa: 1998 (Dapporto et al., 1999).

1775 Capraia: 1980, 1996 (Biermann & Hesch, 1982), authors (2000-2002).

1776 **2006-2015**

1777 Elba: 2006-2015 (authors).

1778 Capraia: 2006-2008, 2010, 2012-2014 (authors).

1779 Giglio: 2007, 2008, 2010, 2012-2014 (authors).

1780 Pianosa: 2011, 2014 (authors).

1781 Montecristo: 2010, 2012, 2014 (authors).

1782 Gorgona: 2015 (authors).

1783

1784 We revealed a strong correlation between the COI structure and the identification of
1785 populations to *L. megera* and *L. paramegaera* (Dapporto 2008) with the individuals
1786 belonging to Capraia and Montecristo clustering with those from Sardinia and Corsica.

1787 According to this diversification this complex of taxa showed a high population
1788 differentiation (Dst 0.615, Gst 0.966, Uni 0.370).
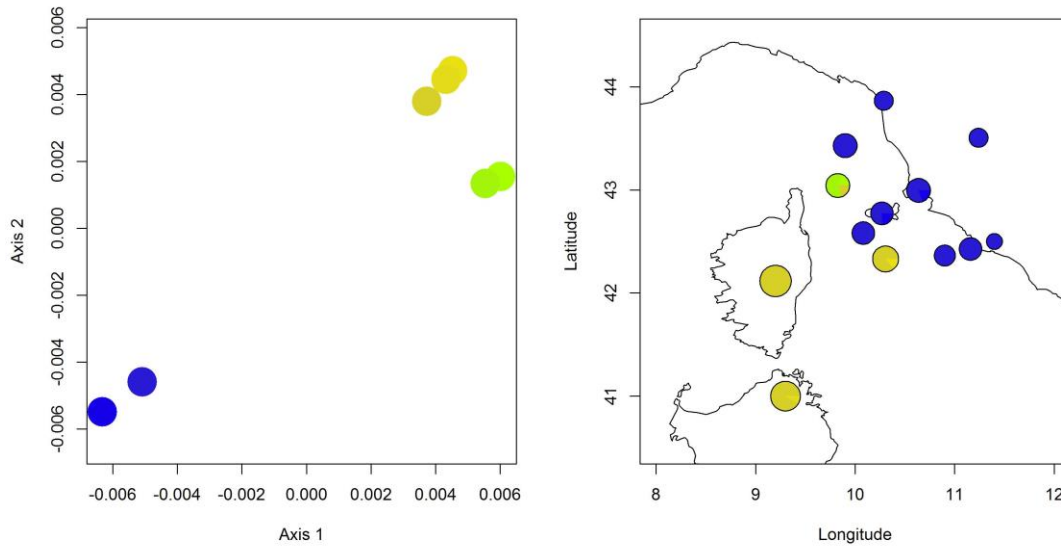
1789

1790

1791 Figure S37. PCoA projection (left) of genetic distances among specimens (dots) in the
1792 bidimensional RGB color space. Individual colours have been subsequently plotted on the
1793 map (right). The first two PCoA axes explained 99.79% of variance.

1794

1795

1796   *Coenonympha corinna* (Hübner, 1804)

1797

1798   The taxonomic status of *Coenonympha corinna* (Sardinia, Corsica, Capraia) and
1799   *Coenonympha elbana* (Elba, Giglio, Giannutri, Argentario and Tuscany coast) has been highly
1800   debated in last years resulting in different authors considering them as different taxa (e.g.
1801   Kodandaramaiah et al. 2009; Balletto et al. 2007) or as a single one (e.g. Dapporto & Strumia
1802   2008; Kudrna et al. 2015). Kodandaramaiah et al. (2009) compared specimens from Sardinia
1803   and Elba for genetic markers finding some diversification while Dapporto and Strumia (2008)
1804   did not reveal any constant morphological diversification in genitalia and a continuous cline
1805   in ocellation.

1806

1807   **1900-2005**

1808   Elba: 1908, 1917, 1920, 1921, 1956, 1957, 1963, 1968, 1980 (Biermann & Hesch, 1982;
1809   Balletto et al. 2007), 1998 (Biermann, 1999), 2000-2003 (authors)

1810   Giglio: 1980 (Biermann & Hesch, 1982), 2000 (Balletto et al. 2007).

1811   Capraia: 1915, 1968, 1978-1980, 1995, 1996 (Biermann & Hesch, 1982; Balletto et al. 2007),
1812   authors (2000-2003).

1813   Giannutri: 1994 (Baletto et al. 2007).

1814   **2006-2015**

1815   Elba: 2008-2010, 2012-2015 (authors).

1816   Capraia: 2006-2008, 2010, 2012-2014 (authors).

1817   Giannutri: 2007, 2014 (authors).

1818

1819   *Not recorded on Giglio in the last 10 years.*

1820

1821   The analysis of a complete COI dataset revealed a complex pattern of diversification with the
1822   existence of three main clades (I. Sardinia, II. Corsica+Capraia, III. Elba+Giannutri+Tuscan
1823   mainland). This diversification did not correlate with the supposed taxonomic diversification
1824   between Sardinia-Corsica-Capraia and Elba-Giannutri-Tuscany coast populations. The

1825 haplotype diversification appeared to be high (with a maximum divergence of 5.2%) and it
1826 revealed a high population diversification and uniqueness (Dst 2.194, Gst 0.943, Uni 0.190).

1827
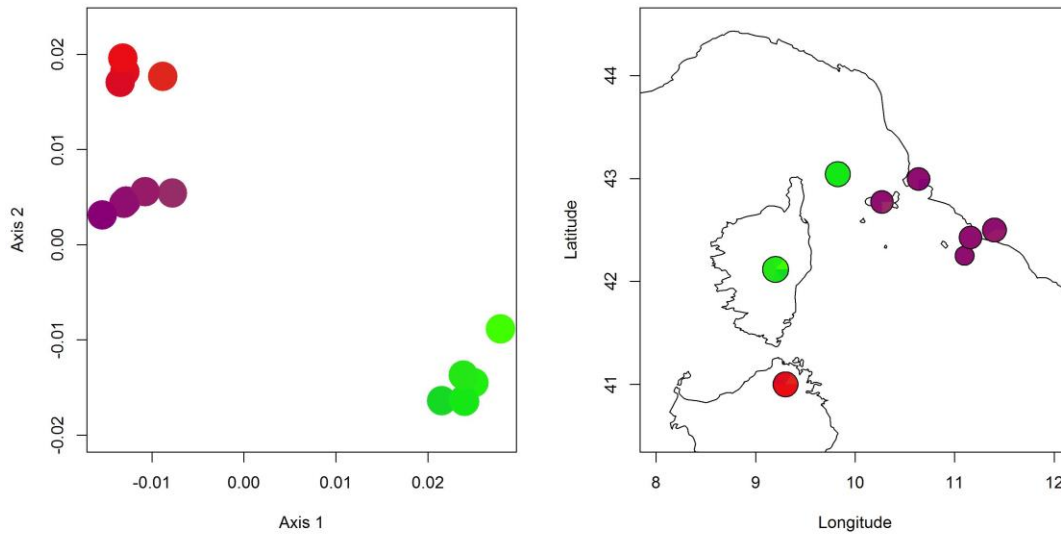
1828

1829

1830 Figure S38. PCoA projection (left) of genetic distances among specimens (dots) in the
1831 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1832 map (right). The first two PCoA axes explained 97.41% of variance.

1833   *Coenonympha pamphilus* (Linnaeus, 1758)

1834

1835   It has been suggested that populations from North Africa, Southern Spain, Balearics and
1836   Sardinia belong to a different species (*Coenonympha lyllus*) (Boillat, 2002). Actually,
1837   populations from North Africa and Balearics differ from those belonging to Europe but the p-
1838   distance in COI are low (Dincă et al. 2015; Vodă et al. 2016). We confirmed here that the
1839   Sardinian specimens belong to the North African clade and that the maximum divergence
1840   was 2.4%, for this reason we analysed the two taxa together.

1841

1842   **1900-2005**

1843   Elba:1908, 1916, 1921, 1963, 1968, 1980 (Biermann & Hesch, 1982; Balletto et al. 2007),
1844   1998 (Biermann, 1999).

1845   **2006-2015**

1846   Elba: 2008-2015.

1847

1848   We revealed a strong correlation between the COI structure and the identification of
1849   populations to *C. pamphilus* and *C. lyllus* (Boillat 2002) with the individuals from Sardinia
1850   representing a different genetic clade from those belonging to other localities. According to
1851   this diversification this taxon showed a high population differentiation (Dst 0.856, Gst
1852   0.864). One haplotype from Elba was not recorded on the mainland resulted in a uniqueness
1853   of 0.152.

1854
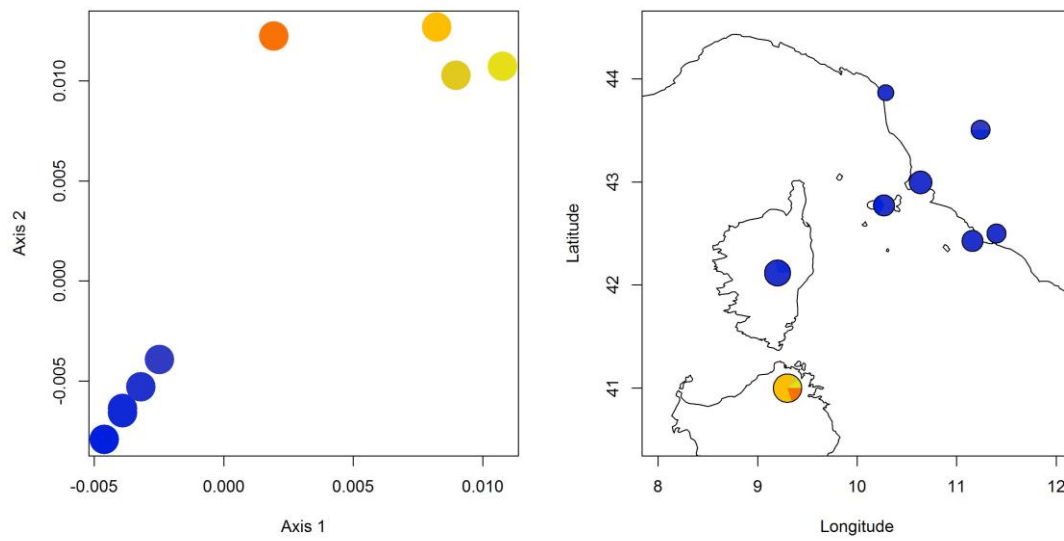
1855

1856

1857 Figure S39. PCoA projection (left) of genetic distances among specimens (dots) in the
1858 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1859 map (right). The first two PCoA axes explained 98.74% of variance.

1860

1861

1862 *Pyronia tithonus* (Linnaeus, 1771)

1863

1864 **1900-2005**

1865 Elba:1908, 1921, 1963, 1968, 1980 (Biermann & Hesch, 1982; Balletto et al. 2007), 2003
1866 (authors).

1867 **2006-2015**

1868 Elba: 2008, 2010, 2012, 2015 (authors).

1869

1870 This species showed a low diversification among haplotypes (maximum p-distance 0.3%),
1871 and their distribution was not spatially structured resulting in low population diversification
1872 (Dst 0.015 and Gst 0.260). The haplotype from Elba also occurs on the mainland.

1873
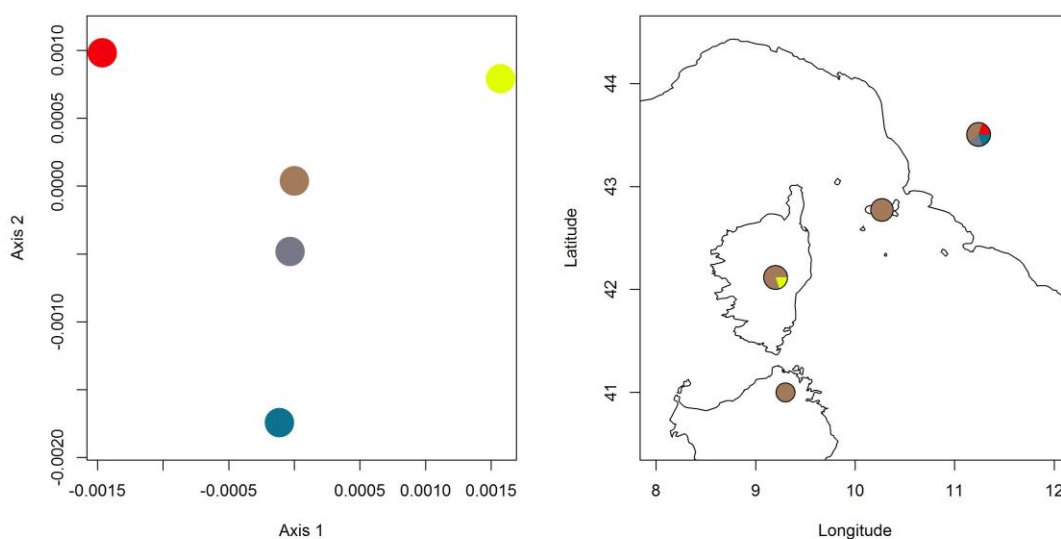
1874

1875

1876 Figure S40. PCoA projection (left) of genetic distances among specimens (dots) in the
1877 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1878 map (right). The first two PCoA axes explained 97.04% of variance.

1879

1880

1881   *Pyronia cecilia* (Vallantin, 1894)

1882

1883   **1900-2005**

1884   Elba: 1908, 1916, 1921, 1956, 1957, 1963, 1968, 1980 (Biermann & Hesch, 1982; Balletto et
1885   al. 2007), 2000-2002 (authors).

1886   Capraia: 1961, 1978, 1996 (Biermann & Hesch, 1982; Balletto et al. 2007), 2001 (authors).

1887   Pianosa: 1998 (Dapporto et al., 1999).

1888   Giglio: 1980 (Biermann & Hesch, 1982), 2003, 2004 (authors).

1889   **2006-2015**

1890   Elba: 2008, 2010, 2012, 2013, 2015 (authors).

1891   Pianosa: 2011 (authors).

1892   Giglio: 2008, 2010 (authors).

1893

1894   *Not recorded on Capraia in the last 10 years.*

1895

1896   As showed by Vodă et al. (2016) this species shows two highly diverging clades, the first
1897   belongs to North Africa, the second to the Italian mainland. The two clades are sympatric in
1898   Sicily. We confirmed the existence of two highly diverging clades also in the study area
1899   (maximum diversification 2.9%). The clades from S-W Mediterranean is the only occurring on
1900   the studied islands, while on the mainland it coexists with the clade typical of the Italian
1901   peninsula. According to this pattern the species showed a high Dst (0.224) but a medium Gst
1902   (0.260). The occurrence on Pianosa and Giglio of haplotypes not found on the mainland
1903   resulted in an island uniqueness value of 0.091.

1904
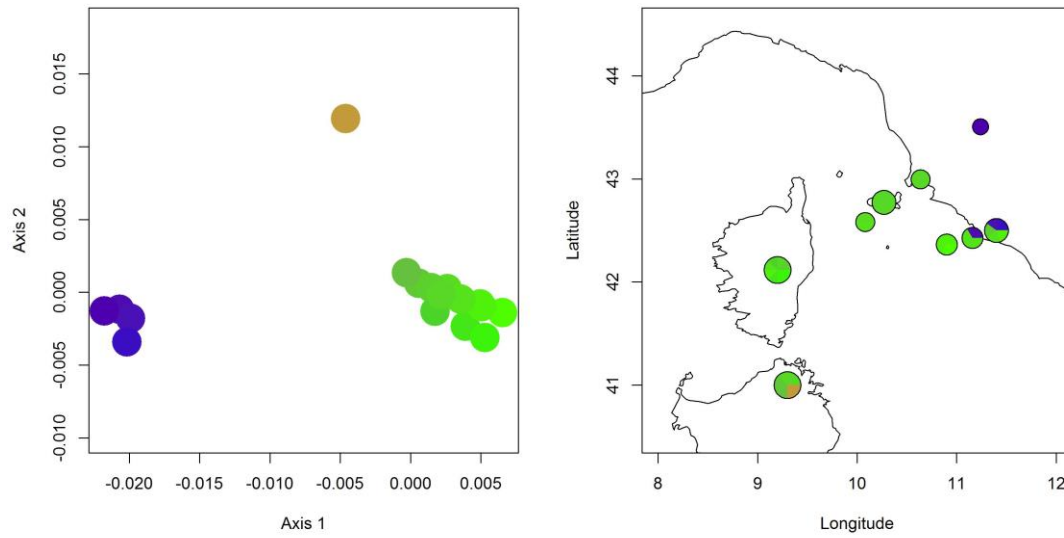
1905

1906

1907 Figure S41. PCoA projection (left) of genetic distances among specimens (dots) in the
1908 bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1909 map (right). The first two PCoA axes explained 85.40% of variance.

1910

1911

1912 *Maniola jurtina* (Linnaeus, 1758)

1913

1914 **1900-2005**

1915 Elba: 1908, 1916, 1919, 1921, 1963, 1967-1970, 1972, 1980, 2000 (Biermann & Hesch, 1982;
1916 Balletto et al. 2007), 2002 (authors).

1917 Capraia: 1915, 1978 (Biermann & Hesch, 1982; Balletto et al. 2007), 1968-1970 (Gross,
1918 1970).

1919 Pianosa: 1998 (Dapporto et al., 1999).

1920 Giglio: 1908, 1967, 1969, 1970, 1979 (Biermann & Hesch, 1982, Balletto et al. 2007), 2001
1921 (authors).

1922 **2006-2015**

1923 Elba: 2008, 2010, 2012, 2013, 2015 (authors).

1924 Pianosa: 2011, 2014 (authors).

1925 Giglio: 2007, 2008, 2010, 2012, 2014 (authors).

1926

1927 It has been recognized since decades that this species shows two main lineages in Europe
1928 differing for morphological and genetic characters (e.g. Thomson 1987; Schmitt et al. 2005;
1929 Dapporto et al. 2009; 2014). The study area is one of the contact and hybrid zones for the
1930 two lineages (Thomson 1987; Dapporto et al. 2014). We confirmed this observation showing
1931 that this species has a maximum divergence of 1.2% in the study area and that the two
1932 lineages coexist on Elba and Corsica with similar frequencies. Accordingly, the parameters
1933 for populations differentiation were high (Dst 0.298, Gst 0.603). The occurrence on Elba and
1934 Pianosa of two haplotypes not found on the mainland resulted in an island uniqueness value
1935 of 0.085.

1936
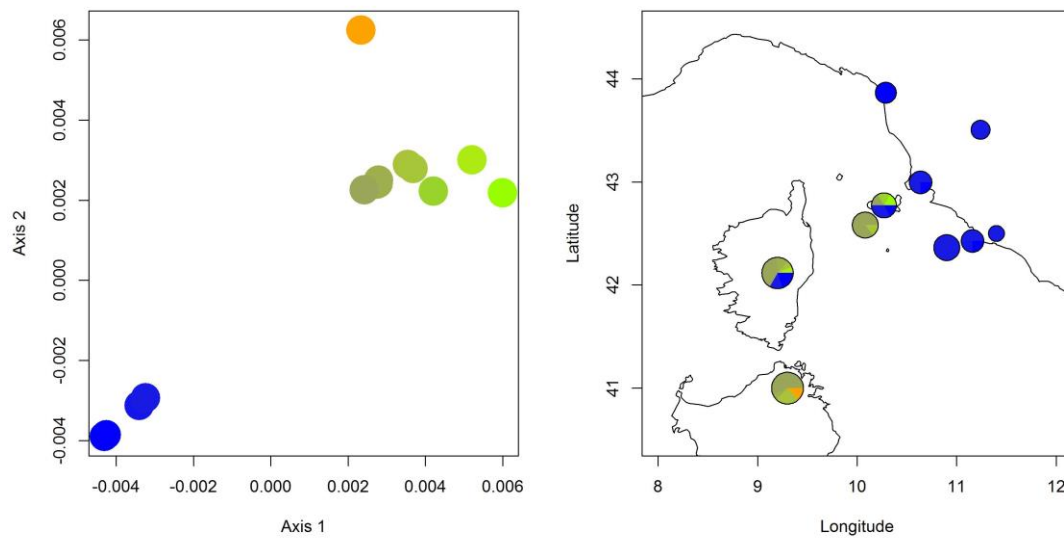
1937

1938

Figure S42. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 93.34% of variance.

1942

1943

1944   *Hipparchia neomiris* (Godart, 1822)

1945

1946   **1900-2005**

1947   Capraia: 1993 (Terzani, 1994).

1948   Elba: 1916, 1917, 1920, 1921, 1963, 1968, 1978, 1979, 1980, 1993, 2002 (Biermann & Hesch,
1949   1982, Balletto et al. 2007), 2002 (Favilli et al., 2003).

1950   **2006-2015**

1951   Elba: 2008-2010, 2012.

1952

1953   *Not recorded in Capraia in the last 10 years.*

1954

1955   This species, endemic of the study area, showed a large diversification (maximum p-distance
1956   2.3%) between two lineages, the first occurring on Sardinia and the second on Corsica and
1957   Elba. A previous analysis on genitalic shape also revealed a diversification among the same
1958   populations (Dapporto 2010), suggesting the need of further studies to understand the
1959   taxonomic status of the two lineages. According to such a clear spatial pattern, the species
1960   showed a high population differentiation (Dst 0.968, Gst 0.871). The status of island endemic
1961   did not allow the calculation of uniqueness.

1962
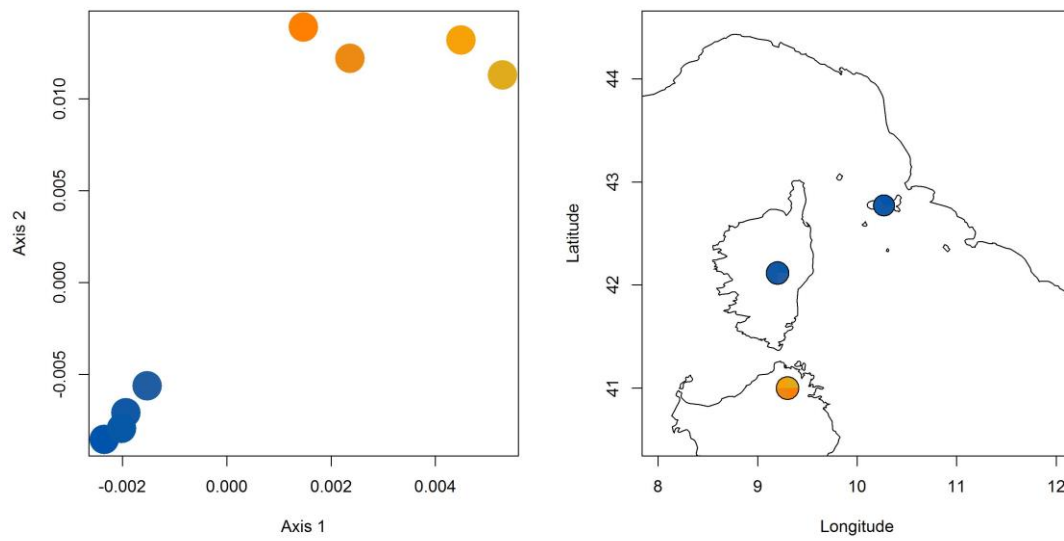
1963

1964

1965　Figure S43.　PCoA projection (left) of genetic distances among specimens (dots) in the
1966　bidimensional RGB colour space. Individual colours have been subsequently plotted on the
1967　map (right). The first two PCoA axes explained 99.85% of variance.

1968

1969

1970   *Hipparchia aristaeus* (Bonelli, 1826)

1971

1972   The taxonomic status of the species belonging to the *H. semele* group is highly debated

1973   mostly on Mediterranean islands (e.g. Cesaroni et al. 1994; Balletto et al. 2007; Dapporto

1974   2010; Kudrna et al. 2015; Vodă et al. 2016). For COI *Hipparchia aristaeus* represents a clearly

1975   distinct clade separated by more than 4% of divergence from *H. semele* and for this reason

1976   we analysed it separately.

1977

1978   **1900-2005**

1979   Gorgona: 1999-2002 (Dapporto, 2002).

1980   Capraia: 1993 (Terzani, 1994).

1981   Elba: 1908, 1920, 1921, 1963, 1968, 1980, 1987, 1988, 2002 (Biermann & Hesch, 1982,

1982   Balletto et al. 2007), 2002 (Favilli et al. 2003), 2000 (authors).

1983   Giglio: 1908, 1979, 1989 (Biermann & Hesch, 1982, Balletto et al. 2007).

1984   **2006-2015**

1985   Elba: 2008-2010, 2012, 2013, 2015 (authors).

1986   Giglio: 2010, 2013, 2014 (authors).

1987

1988   This species showed a very low genetic variation over the study area (maximum p-distance

1989   0.6%) and almost no spatial structure (Dst 0.019, Gst 0.119). The status of island endemic did

1990   not allow the calculation of uniqueness
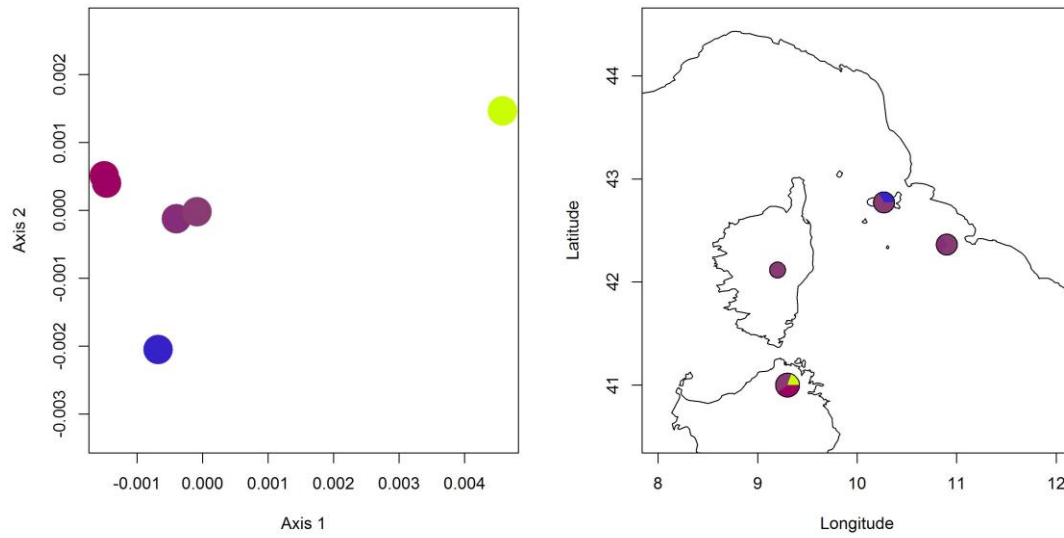
1991

1992

Figure S44. PCoA projection (left) of genetic distances among specimens (dots) in the bidimensional RGB colour space. Individual colours have been subsequently plotted on the map (right). The first two PCoA axes explained 87.53% of variance.

1996

1997

1998   *Hipparchia statilinus* (Hufnagel, 1766)

1999

2000   **1900-2005**

2001   Elba: 1908, 1916, 1917, 1920, 1963, 1980 (Biermann & Hesch, 1982, Balletto et al. 2007).

2002   **2006-2015**

2003   Elba: 2009, 2012, 2013 (authors).

2004

2005   This species showed a low diversification among haplotypes (maximum p-distance 0.5%),
2006   and their distribution was slightly spatially structured resulting in low population
2007   diversification (Dst 0.053 and Gst 0.281). The haplotypes occurring on Elba have been also
2008   found on the mainland.

2009
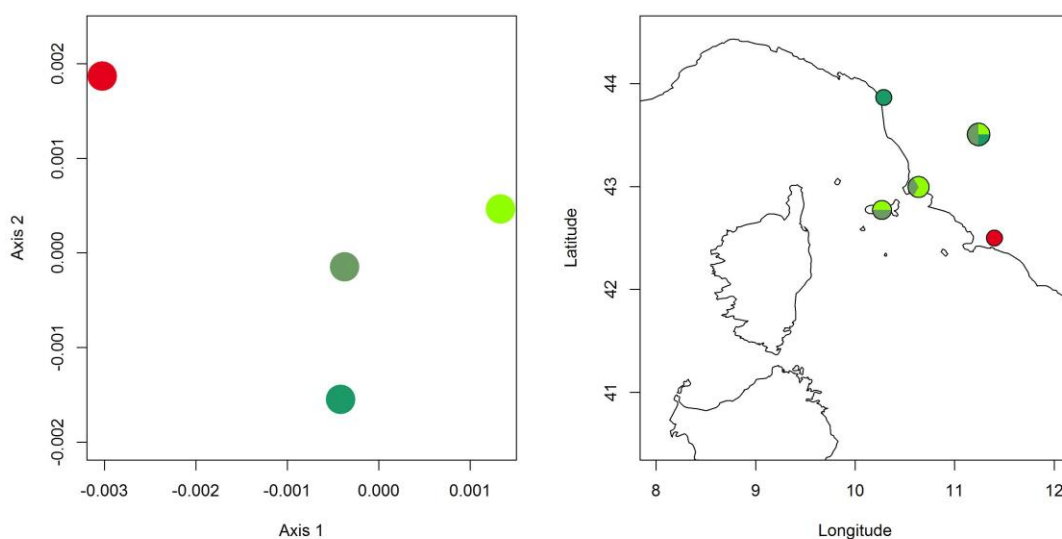
2010

2011

2012   Figure S45. PCoA projection (left) of genetic distances among specimens (dots) in the
2013   bidimensional RGB colour space. Individual colours have been subsequently plotted on the
2014   map (right). The first two PCoA axes explained 85.09% of variance.

2015

2016

2017

2018   **Supplementary results**

2019
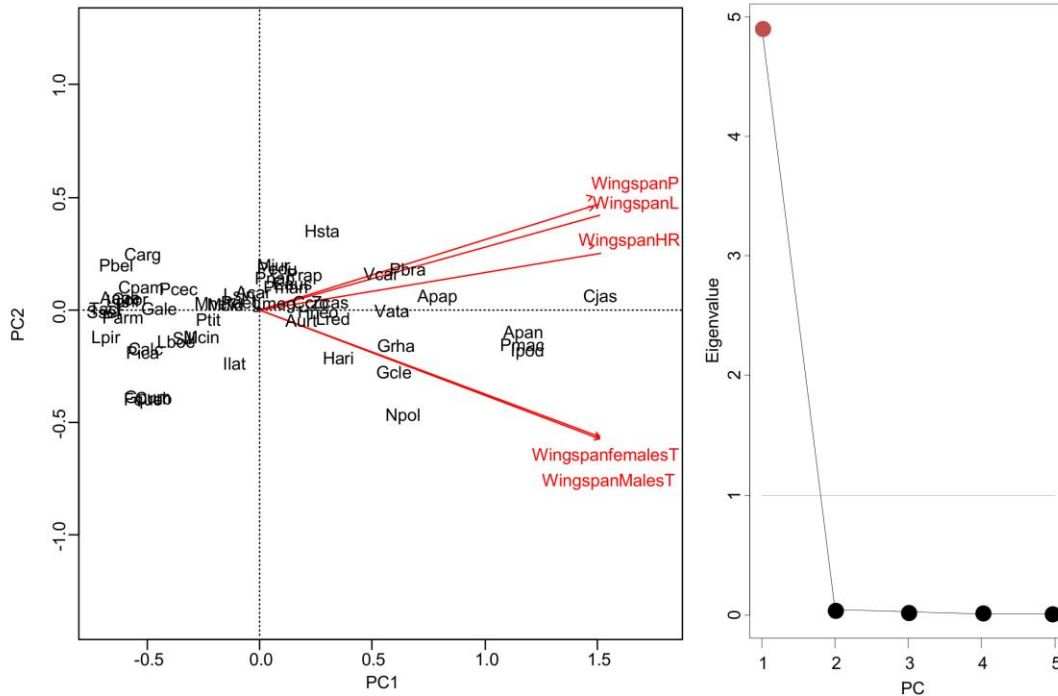
2020

2021

2022   Figure S46. PCA scatterplot for wingspan (left). Data belong to four literature sources
2023   WingspanP (Pamperis, 2009), WingspanL (Lafranchis, 2000), WingspanHR (Higgins & Riley,
2024   1970), WingspanfemalesT female data from Tshikolovets (2011), WingspanmalesT male data
2025   from Tshikolovets (2011). Tags for butterflies are obtained by using the first letter of the
2026   genus name and the first three letters of the species name. The eigenvalue plot for the five
2027   components (right) shows that only the first PC (red dot) had an eigenvalue higher than 1.

2028

2029

Figure S47. PCA scatterplot for phenology traits (left). Tags for butterflies are obtained by using the first letter of the genus name and the first three letters of the species name. The eigenvalue plot for the five components (right) shows that the first two PCs (red dots) had an eigenvalue higher than 1.

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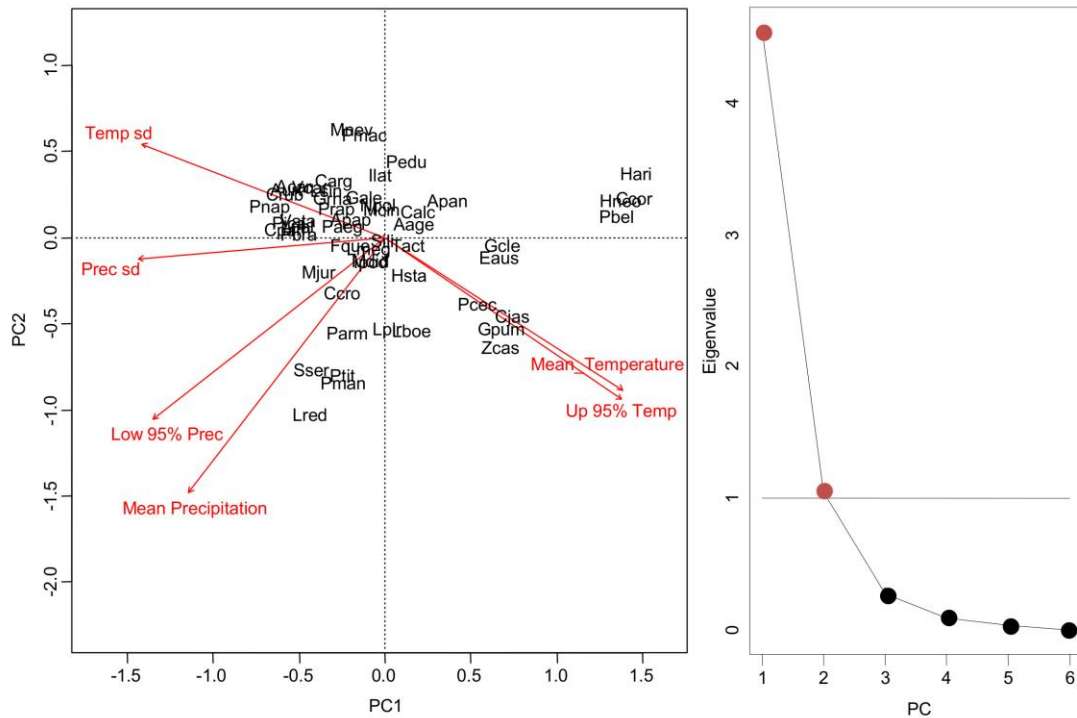
2036

Figure S48. PCA scatterplot for physiological traits (left). Tags for butterflies are obtained by
using the first letter of the genus name and the first three letters of the species name. The
eigenvalue plot for the five components (right) shows that the first two PCs (red dots) had an
eigenvalue higher than 1.

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	Host_Plants				
MobilityPC1	-0.168	MobilityPC1			
PhenolPC1	0.277	0.119	PhenolPC1		
PhenolPC2	0.277	-0.200	0.000	PhenolPC2	
EcophyPC1	-0.351	0.048	-0.308	-0.114	EcophyPC1
EcophyPC2	0.090	0.109	-0.021	0.073	0.000

2046

2047　Table S1. Correlations among host plants and the six traits obtained after Principal
2048　Component Analysis.

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Melanargia_galathea
Melanargia_russiae
Melanargia_pherusa
Melanargia_arge
Maniola_jurtina
Maniola_nurag
Pyronia_cecilia
Pyronia_tithonus
Chazara_briseis
Brintesia_circe
Coenonympha_pamphilus
Coenonympha_lyllus
Coenonympha_corinna
Hyponephele_lycaon
Hyponephele_lupina
Hipparchia_fagi
Hipparchia_hermione
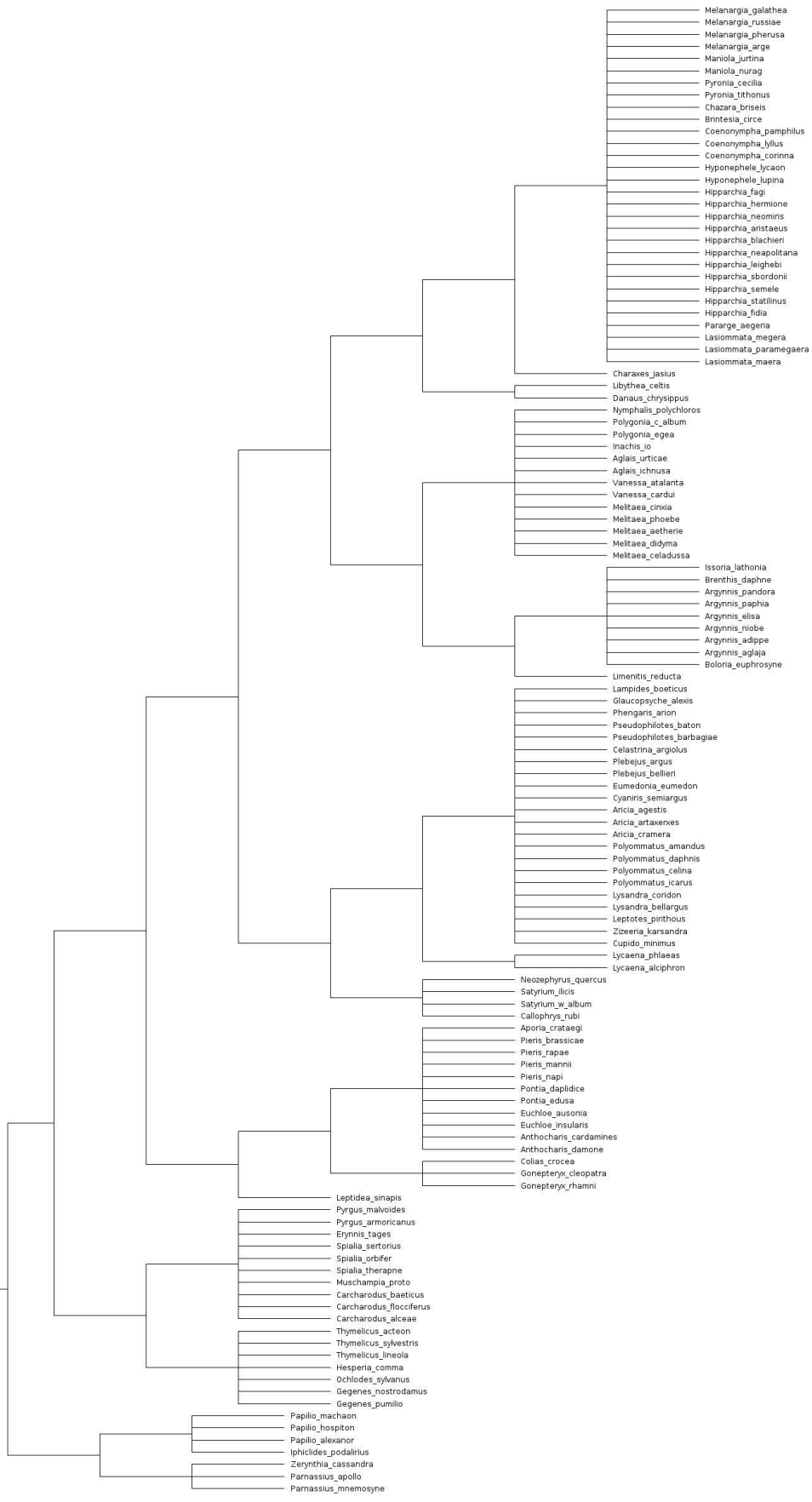Hipparchia_neomiris
Hipparchia_aristaeus
Hipparchia_blachieri
Hipparchia_neapolitana
Hipparchia_leighebi
Hipparchia_sbordonii
Hipparchia_semele
Hipparchia_statilinus
Hipparchia_fidia
Pararge_aegeria
Lasiommata_megera
Lasiommata_paramegaera
Lasiommata_maera
Charaxes_jasius
Libythea_celtis
Danaus_chrysippus
Nymphalis_polychloros
Polygonia_c_album
Polygonia_egea
Inachis_io
Aglais_urticae
Aglais_ichnusa
Vanessa_atalanta
Vanessa_cardui
Melitaea_cinxia
Melitaea_phoebe
Melitaea_aetherie
Melitaea_didyma
Melitaea_celadussa
Issoria_lathonia
Brenthis_daphne
Argynnis_pandora
Argynnis_paphia
Argynnis_elisa
Argynnis_niobe
Argynnis_adippe
Argynnis_aglaja
Boloria_euphrosyne
Limenitis_reducta
Lampides_boeticus
Glaucopsyche_alexis
Phengaris_arion
Pseudophilotes_baton
Pseudophilotes_barbagiae
Celastrina_argiolus
Plebejus_argus
Plebejus_bellieri
Eumedonia_eumedon
Cyaniris_semiargus
Aricia_agestis
Aricia_artaxerxes
Aricia_cramera
Polyommatus_amandus
Polyommatus_daphnis
Polyommatus_celina
Polyommatus_icarus
Lysandra_coridon
Lysandra_bellargus
Leptotes_pirithous
Zizeeria_karsandra
Cupido_minimus
Lycaena_phlaeas
Lycaena_alciphron
Neozephyrus_quercus
Satyrium_ilicis
Satyrium_w_album
Callophrys_rubi
Aporia_crataegi
Pieris_brassicae
Pieris_rapae
Pieris_mannii
Pieris_napi
Pontia_daplidice
Pontia_edusa
Euchloe_ausonia
Euchloe_insularis
Anthocharis_cardamines
Anthocharis_damone
Colias_crocea
Gonepteryx_cleopatra
Gonepteryx_rhamni
Leptidea_sinapis
Pyrgus_malvoides
Pyrgus_armoricanus
Erynnis_tages
Spialia_sertorius
Spialia_orbifer
Spialia_therapne
Muschampia_proto
Carcharodus_baeticus
Carcharodus_flocciferus
Carcharodus_alceae
Thymelicus_acteon
Thymelicus_sylvestris
Thymelicus_lineola
Hesperia_comma
Ochlodes_sylvanus
Gegenes_nostrodamus
Gegenes_pumilio
Papilio_machaon
Papilio_hospiton
Papilio_alexanor
Iphiclides_podalirius
Zerynthia_cassandra
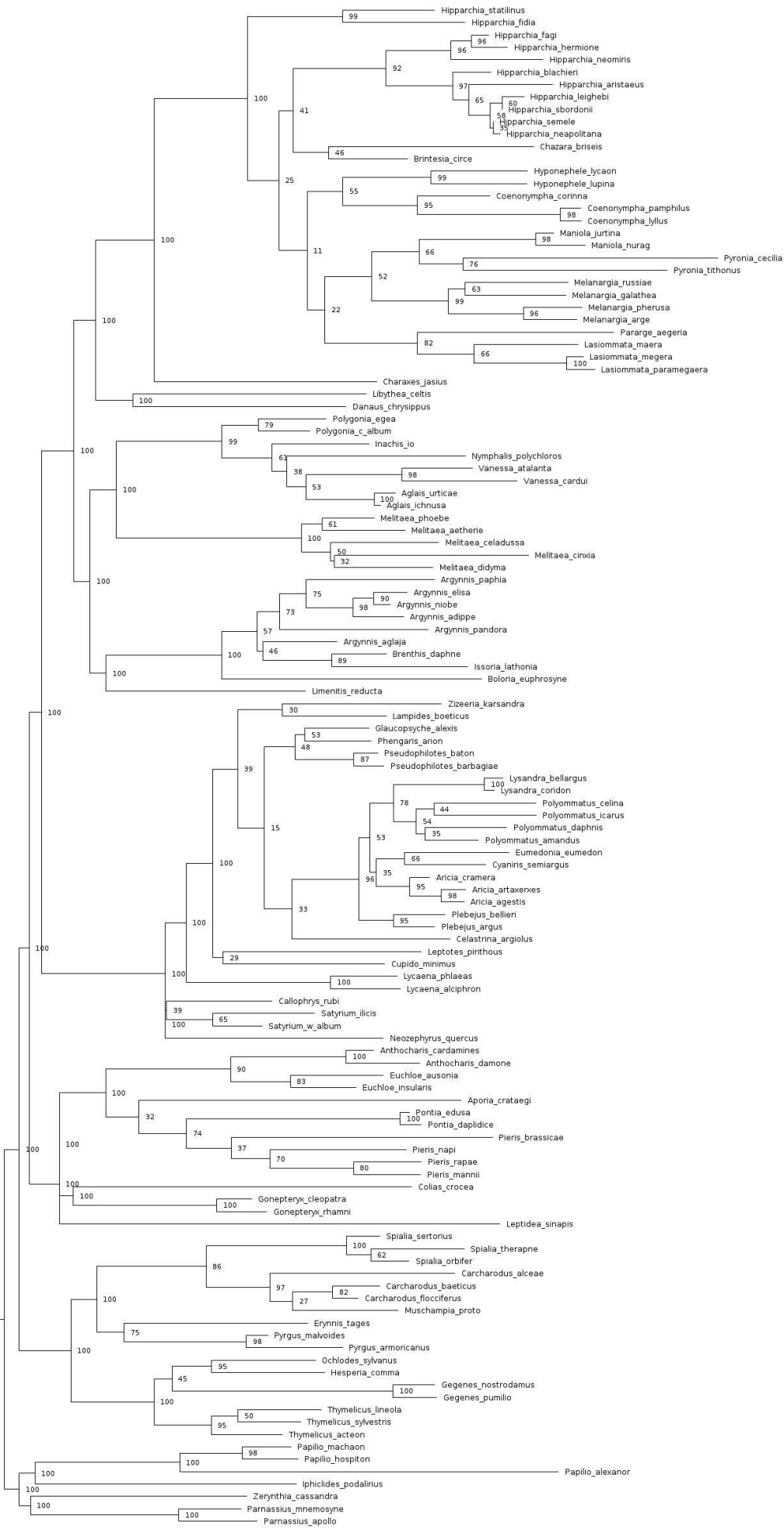Parnassius_apollo
Parnassius_mnemosyne

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0.9

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2071   Figure S49. The topological constraints at family and subfamily levels applied to the COI
2072   phylogenetic tree based following the butterfly phylogeny published in Heikkila et al. (2012).
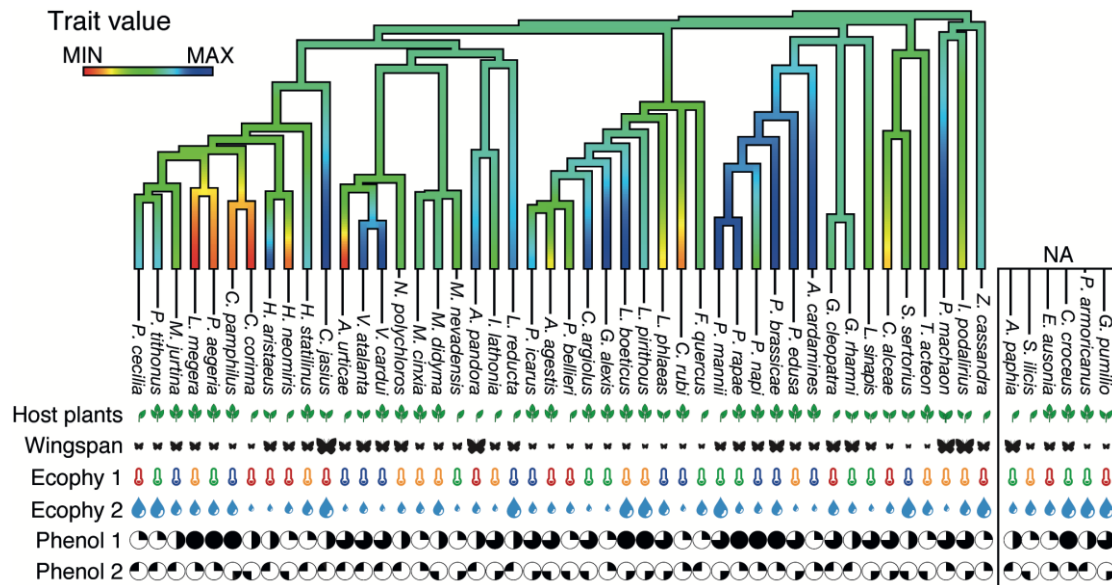
2073

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2079   Figure S50. Supports for nodes in the phylogenetic tree used in phylogenetic regression.
2080   Family and subfamily nodes costrained according to Heikkila et al. (2012) are by default
2081   characterized by a maxium support.

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2088 Figure S51 Gst values for species mapped over the phylogenetic tree based on cytochrome *c*
2089 oxidase subunit 1 (COI) tree. Host plants represent the number of plant genera on which the
2090 larva of a given species has been reported: one leaf - one genus; two leaves two to four
2091 genera, three leaves more than four genera. The sizes of the butterfly silhouettes are
2092 directly correlated with the species size (wingspan). Ecophy 1 represents the first PC of
2093 physiology traits mostly represent mean temperature and is reported with colours
2094 representing quartiles of values (red, preference for warm temperatures; blue, preference
2095 for cold temperatures). Ecophy 2 represents the second PC of physiology traits mostly
2096 correlated with precipitation tolerance and represented in quartile from small (high
2097 tolerance to drought) to large drops (low tolerance to drought). Phenol 1 represent quartiles
2098 the first PC of phenology correlated with the length of flying period from shortest (one black
2099 sector) to longest (four black sectors). Phenol 2, mostly linked to the period of emergence,
2100 represent spring species appearing early in the year (black sector right-top) to species with a
2101 later summer-autumn appearance (black sector left-top).
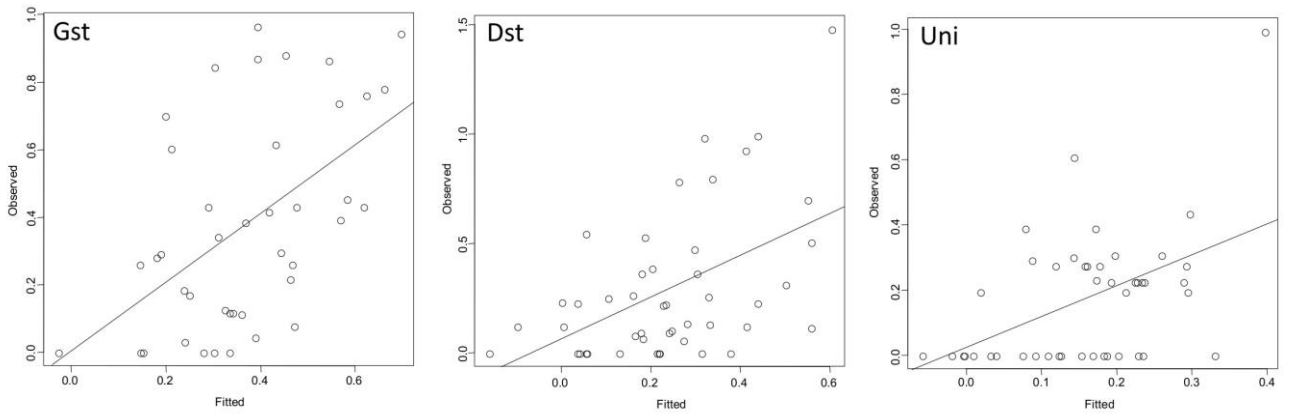
2102

2103
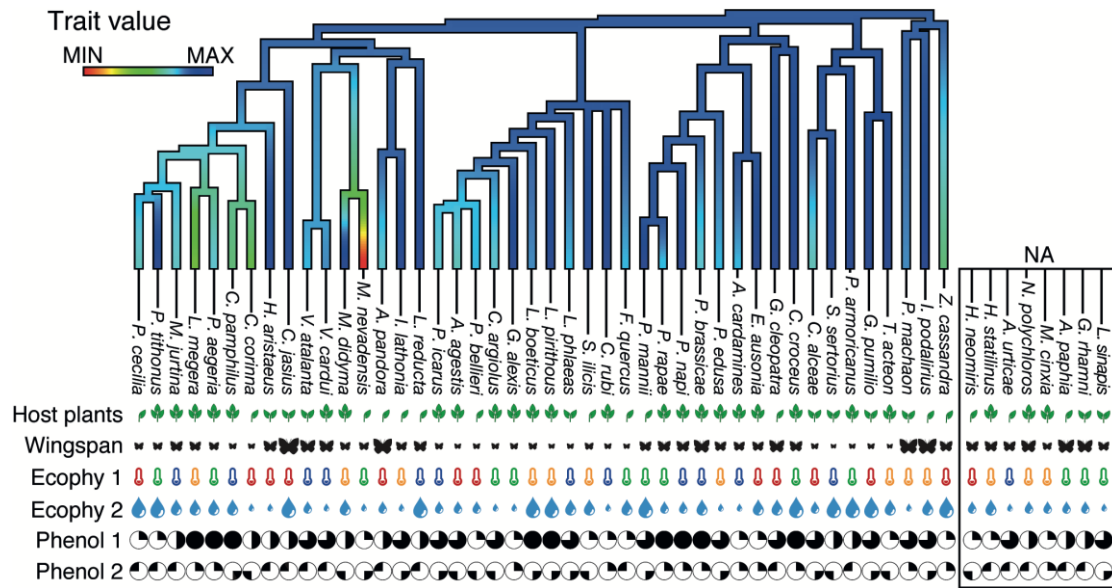
2104

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2106   Figure S52 Uniqueness values for species mapped over the phylogenetic tree based on
2107   cytochrome *c* oxidase subunit 1 (COI) tree. Symbols for traits as in the previous figure.
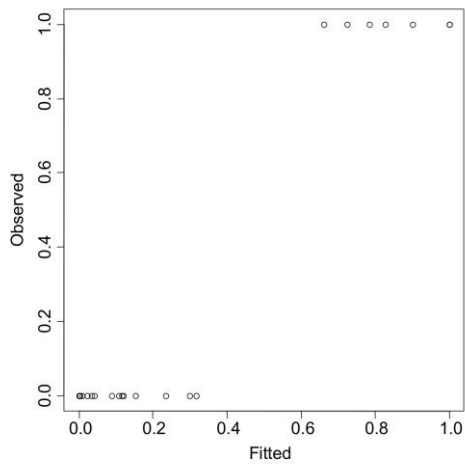
2108

2109

2110   Figure S53. Plots for observed vs fitted values in phylogenetic multiple regression models for

2111   Gst, Dst and uniqueness.

2112

2113

2114   Figure S54. Plot for observed vs fitted values in phylogenetic generalized linear model for

2115   extinction in Capraia.

2116

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2127 In the main paper, we recognised as separate units most species accepted by the Fauna
2128 Europaea Project (Karsholt & Van Nieukerken, 2013; www.faunaeur.org) and considered as
2129 single unit taxa displaying a minimum COI p-distance lower than 3%. We re-performed all
2130 the analyses without setting any threshold. In this case we used the Fauna Europea
2131 taxonomy to identify units. The results turned out to be very similar and they are reported
2132 below:

2133

	Gst	
Dst	0.790***	Dst
Uni	0.103 n.s.	0.385**

2134

2135 Table S2. Spearman correlation between Gst, Dst and Uni

2136

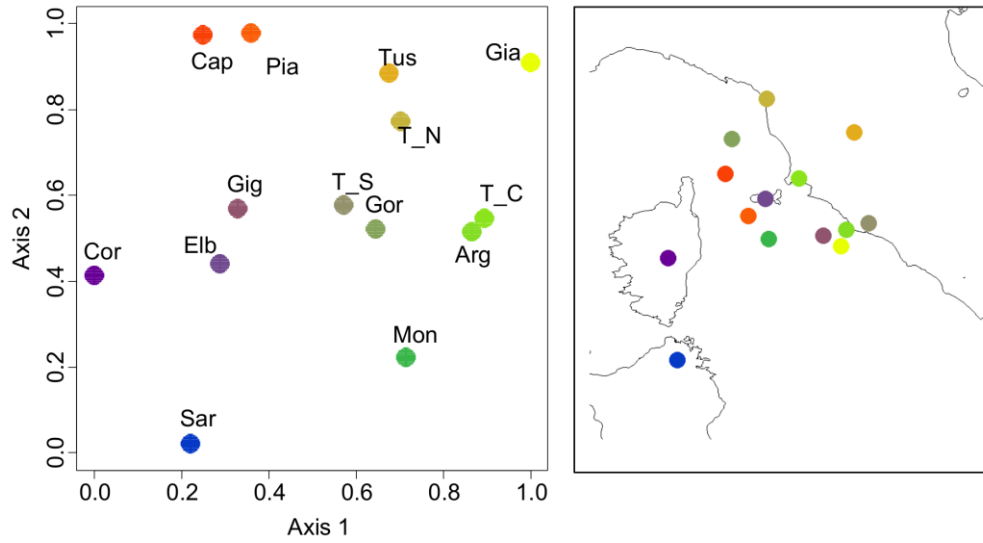| Index	Pagel's lambda	p
Gst	0.136	0.519
Dst	0.342	0.053
Uni	0.050	0.709

2137

2138 Table S3. Phylogenetic signal for Gst, Dst and Uni.

2139

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Figure S55. Overall genetic patterns obtained after comparisons among islands based on Gst. (left) The colours obtained in the RGB projection of the Principal Coordinate Analyses have been included in (right) the map of the studied region to show similarity and contrast among areas. The locality codes are: Arg, Argentario; Cap, Capraia; Cor, Corsica; Elb, Elba; Gia, Giannutri; Gig, Giglio; Gor, Gorgona; Mon, Montecristo; Pia, Pianosa; Sar, Sardinia; Tus, Tuscany; T_C central Tuscany coast; T_N, northern Tuscany coast; T_S, southern Tuscany coast. Protest between PCoA configuration and spatial location, Procrustes correlation 0.641, P=0.006.

2151

2152

	Estimate	StdErr	t	p
Gst				
Host_Plants	-0.076	0.040	-1.880	0.068
MobilityPC1	-0.126	0.042	-2.903	0.006
EcophysiolPC2	0.066	0.038	1.769	0.085
Dst				
Host_Plants	-0.080	0.088	-2.520	0.016
MobilityPC1	-0.098	0.048	-2.040	0.048
EcophysiolPC2	0.122	0.032	3.860	<0.001
Uniqueness				
Host_Plants	-0.048	0.026	-1.843	0.073
EcophysiolPC2	0.093	0.025	3.680	<0.001

2153

2154

2155

2156 Table S4 Trait variables entered in the AIC phylogenetic regressions for Gst, Dst and
2157 Uniqueness (Uni).

2158

	Estimate	StdErr	z.value	p.value
Elba				

Host_Plants	-0.383	0.467	-0.819	0.413
MobilityPC1	0.410	0.481	0.851	0.395
PhenologyPC1	-0.401	0.555	-0.723	0.469
PhenologyPC2	-0.521	0.438	-1.190	0.234
EcophysiolPC1	-0.843	0.622	-1.355	0.176
EcophysiolPC2	0.790	0.568	1.392	0.164
Capraia				
Host_Plants	-0.969	0.974	-0.995	0.320
MobilityPC1	1.628	1.216	1.339	0.181
PhenologyPC1	-6.470	3.055	-2.118	0.034
PhenologyPC2	2.906	1.905	1.524	0.127
EcophysiolPC1	-1.877	1.124	-1.508	0.132
EcophysiolPC2	2.765	1.789	1.545	0.122

2159

2160 Table S5. Species traits associated to disappearance on Elba and Capraia based on
2161 phylogenetic GLM with logistic model.

2162

2163

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