

Invading a refugium: post glacial replacement of the ancestral lineage of a Nymphalid butterfly in the West Mediterranean

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Abstract The power of a fixed scenario was compared to that of a dynamic scenario, in order to explain the distribution pattern of two well established subspecies (morphotypes) of the model butterfly species *Maniola jurtina* in the central Mediterranean. Samples were collected from a transect of 21 sites along the western side of the Italian Peninsula as well as in Sicily, North Africa and some Italian islets in the Tyrrhenian sea. Samples from the Balkans Peninsula have been added for comparison. Geometric morphometrics, suggested as a reliable marker to identify hybrid individuals, were applied to 150 male genitalia. Their shape was analysed by the means of the partial least square (PLS) discriminant analysis and then modelled through geographic information system (GIS) spatial analyses. The timing of invasion was reconstructed by comparing sea-level changes with the recent isobaths both on the mainland and on islands. The occurrence of the eastern morphotype on the Italian Peninsula and of the western morphotype in North Africa and Sicily was confirmed. However, we found intermediate populations at the tip of the Italian Peninsula and on the islands of Ischia and Capri. No intermediate populations were found in Sicily. The fixed scenario is unlikely, since a dispersal of the western morphotype from Sicily to the distant islands of Ischia and Capri might be hypothesized. A more parsimonious hypothesis minimises dispersal across the sea barriers. It assumes the ancestral presence over the entire study area of

the western morphotype, which was later replaced on the Italian mainland but maintained on the islands. These rapid movements could drastically modify European biogeographic patterns.

Keywords Biogeography · Europe · North Africa · GIS · Shorelines reconstruction · *Maniola jurtina*

Introduction

There is strong evidence that climate oscillations, which have been occurring since the early Pleistocene, are the main factors determining the observed biogeography of many organisms (Hewitt 1996, 1999, 2000; Taberlet et al. 1998; Parmesan et al. 1999; Schmitt 2007). Species adapted to warm and temperate climates are thought to have reduced the areas they occupied to Southern refugia during glacial maxima; in these isolated areas, populations experienced genetic differentiation, which resulted in the emergence of different conspecific lineages in many organisms (Hewitt 1999, 2000). During the following warm periods these populations expanded northward and re-occupied their former interglacial areals (Hewitt 1999, 2000; Schmitt 2007). A widely accepted model suggests that this expansion was rapid, as it acted mainly via long-distance dispersal toward empty areas followed by exponential population growth (Hewitt 2000). As a corollary of the edge expansion model, Hewitt (2000) suggested that, after lineages had completely filled the space from different glacial refugia, their relative distributions should have remained essentially stable.

The climate and environmental changes that have occurred at a global scale in recent decades have revealed that the displacements of organism distributions may rapidly follow environmental perturbations (Parmesan et al. 1999;

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Hill et al. 2002; Nogués-Bravo 2009; Fisher et al. 2010). The rapid variations in species distribution due to climate changes suggest that also minor natural environmental changes, which occurred after the deglaciation that began 18 ka BP (Willis and Birks 2006; Sommer and Zachos 2009), might have produced biogeographic effects. Such events might have operated also in very recent times, such as during the so-called “little ice age”, which occurred between the sixteenth and the nineteenth centuries (Lamb 1972). Due to their relatively minor range and duration compared with the major glacial-post glacial cycles, recent climate changes are thought to have produced subtle variations in species distribution and to be evident on a smaller scale (e.g. Schmitt et al. 2005a for a Nymphalidae butterfly). Compared with such recent and subtle events, data derived from fossils are inadequate because of their sparse distribution (Sommer and Zachos 2009). Moreover, fossil data are unavailable for many terrestrial invertebrates, hence past distributions can be reconstructed on the basis of phylogeography and of retrodictive models (see Avise 2000, 2009; Provan and Bennett 2008; Nogués-Bravo 2009; Dapporto et al. 2009, 2011a, b; Habel et al. 2009, 2010, 2011; Dapporto 2010a; Dincă et al. 2011 for Mediterranean butterflies).

The meadow brown butterfly *Maniola jurtina* (Linnaeus, 1758) represents a model species in several studies on phylogeography and speciation, since it forms conspicuous populations along the Mediterranean basins including many small and large islands (Schmitt et al. 2005b; Habel et al. 2009; Dapporto et al. 2009). This species, belonging to the Satyrinae subfamily of the Nymphalidae, is distributed from the Mediterranean area and Central-Northern Europe to the Eastern side of the Caspian sea, also including Iran and the Canary islands (Thomson 2011). The larva feed on a large variety of grasses belonging to several genera (e.g. *Poa*, *Lolium*, *Festuca*, *Bromus*, *Brachypodium*). This species has been considered as a model species in a number of studies on dispersal that revealed that *M. jurtina* is able to move across different suitable patches and that most individuals show high site fidelity (e.g. Conradt et al. 2000; Ouin et al. 2008). Two lineages, identified on the basis of male genitalia and allozyme analysis (Thomson 1987, 2011), exist in Europe: a western Atlantic-Mediterranean lineage (*Maniola jurtina jurtina*) occurring in Maghreb, Spain, western France, Sicily and Sardinia; and an eastern-Asian-Mediterranean lineage (*Maniola jurtina janira*) widespread from Asia to eastern and central Europe including the Italian Peninsula (Thomson 1987; Dapporto et al. 2009). The diversification between the two lineages inhabiting Europe might have occurred during late Pleistocene (last stadial, 40 ka BP, Schmitt et al. 2005b); therefore, they are thought to have experienced a series of contraction/expansion cycles during the following glacial-interglacial periods (Thomson 1987; Dapporto et al. 2009, 2011a; Habel et al.

2009; Nicholls et al. 2010). Morphometric and genetic markers also revealed the presence of a hybrid zone extending from some western Mediterranean islands (Corsica, Elba, Giglio, Capri) to the western Alps and the Benelux region (Thomson 1987, 2011; Schmitt et al. 2005b; Dapporto et al. 2009). By modelling morphological variation over the western Mediterranean area, Dapporto et al. (2009) and Dapporto (2010a) suggested that the western morphotype was once widespread in the whole western Mediterranean mainland and islands, thus including the Italian Peninsula. On the basis of these models, Dapporto et al. (2009, 2011a) suggested that, during one of the post-glacial periods, *M. j. janira* expanded from eastern Europe and invaded the Italian Peninsula, where it replaced the original *M. j. jurtina*. However, even narrow sea straits separating the Italian islets (Corsica, Elba, Giglio, Capri, Sicily) presumably represented barriers that allowed the maintenance of the supposed ancestral *M. j. jurtina* (Dapporto et al. 2009). Unlike in Italy, *M. j. janira* seems to be the ancestral morphotype in the Balkans, as shown by its exclusive occurrence on the islands surrounding the Balkan peninsula (Thomson 1987; L. Dapporto, unpublished data for Zakynthos island). The assessment of such a complex colonisation route clearly contrasts with the main model, which assumes that, after their post-glacial establishment, genetic lineages tend to maintain their relative distributions.

However, in Dapporto et al. (2009), specimens collected from mainland sites were pooled to represent five geographically relevant areas (North Africa, Spain, France, northern Italy and southern Italy). Such a low resolution sampling allowed the formulation of the above mentioned hypothesis, but impeded any further validation at a finer scale. For this reason, in the present paper we searched for empirical evidence of a recent shift in distribution of the two morphotypes using a high resolution data set that included 21 populations occurring in a 1,100 km long transect (from central Italy to Tunisia, including several Sicilian sites as well as the islands of Capri, Ischia and Lipari). We examined the observed distribution of the morphotypes, the dislocation of the areas showing intermediate populations and their relationships with the sea channels. Two alternative hypotheses were considered:

- (1) The western and the eastern morphotypes rapidly occupied their recent distributions during the immediate post-glacial period, when they encountered some well-defined geographic barriers where they created stable hybridization areas (Hewitt 1996; 2000; Schmitt et al. 2005b). If this holds true, we should find areas showing intermediate morphotypes in correspondence to both sides of physical barriers such as sea straits. Moreover, the sea straits established after the dispersal events would not separate any different populations.

- (2) The western morphotype was ancestral in Italy during the glacial and immediate post-glacial phases and only much later, after the current sea channels between the islands and the Italian mainland were restored, did the eastern morphotype invade Italy and replace the ancestral western morphotype (Dapporto et al. 2009, 2011a; Dapporto 2010a). In this case, we expect to find asymmetrical hybrid areas in correspondence to barriers (sea straits). Moreover, sea straits established in relatively recent times should show different populations at their margins as a consequence of relictuality phenomena.

Methods

Study area, sampling and genitalia preparation

A total of 150 males were examined within 21 mainland and insular areas representing a transect from the central Italian Peninsula to western Tunisia, also including four islands (Ischia, Capri, Lipari and Sicily; Fig. 1). The sample also comprises specimens from Balkans Peninsula to validate our hypotheses. The areas were identified as groups of sites located in the same 100 km² UTM grid zone (see Table 1 and Fig. 1 for details). No geographical barriers exist in the study area along

the Italian Peninsula and Tunisia, while sea straits of different length separate the islands from the mainland areas. Ischia is separated from the mainland by two sea straits of about 2 km each, due to the presence of the island of Procida in the middle (Fig. 4b). In contrast, Capri is isolated by a sea strait of about 5 km (Fig. 4a). Sicily is separated from Italian mainland by the Strait of Messina, which has a minimum width of 3 km and is 150 km distant from Tunisia. Finally, Lipari is separated from Sicily by a 30 km strait. Genitalia were dissected by using standard procedures (Dapporto 2008). Abdomens were boiled in 10% caustic potash. Genitalia were cleaned and the left valva and the aedeagus were removed. The tegumen and the right valva were mounted in euparal between microscope slides and cover slips. Genitalia were photographed by using a Nikon Coolpix 4500 camera mounted on a binocular microscope.

Geometric morphometric

The morphometric and statistical approach used was an improved version of that used for the same species by Dapporto et al. (2009). A combination of landmarks and sliding semi-landmarks (Bookstein 1997) was applied by using the thin-plate spline (TPS) series of programs (available at <http://life.bio.sunysb.edu/morph/>)—a method allowing quantitative explorations of shapes. The lateral sections of the brachium, the valva and the tegumen were examined. Three points on the

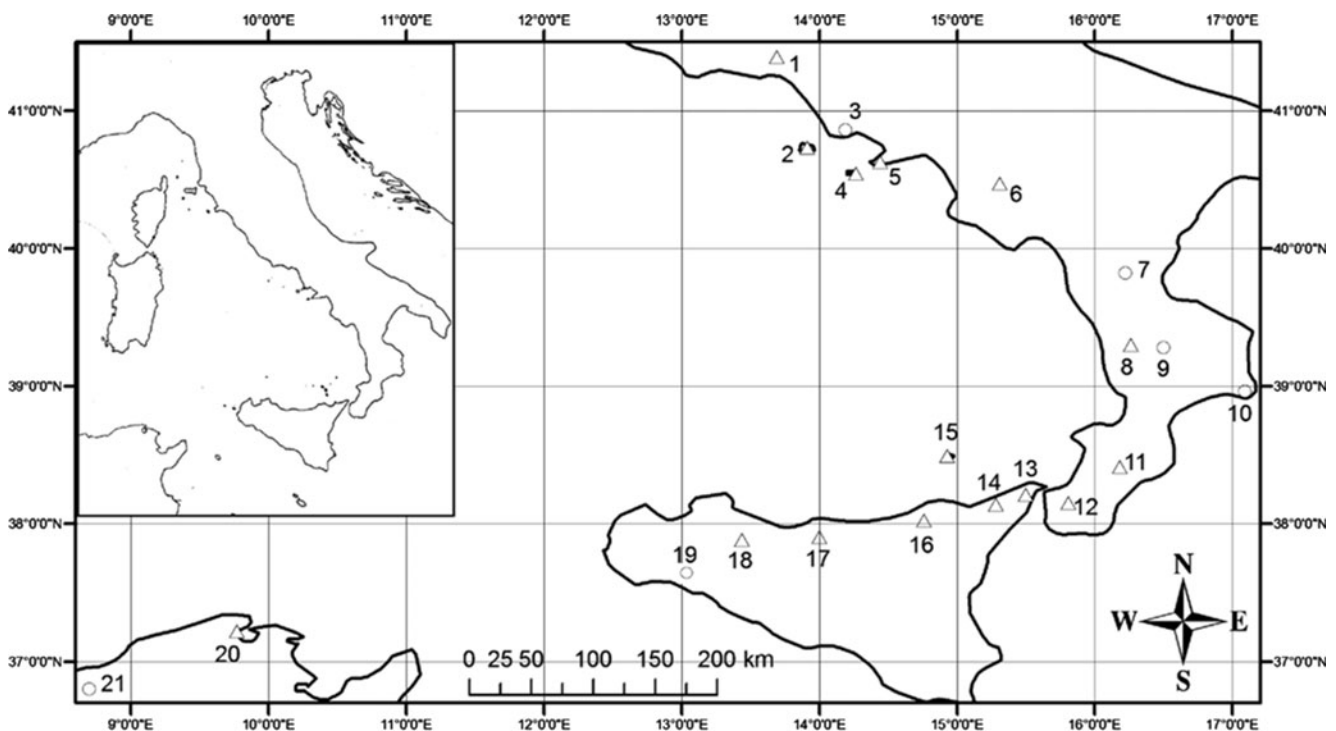


Fig. 1 The study area showing the 21 sampled localities: 1 Esperia, 2 Ischia, 3 Napoli, 4 Capri, 5 Punta Campanella, 6 Ottati, 7 Castrovillari, 8 Cosenza, 9 Sila Grande, 10 Isola Capo Rizzuto, 11 Monte Limina, 12 Gambarie, 13 Portella Rizzo, 14 Santa Lucia sul Melo, 15 Lipari, 16 Galati

Mamertino, 17 Madonie, 18 Ficuzza, 19 Lago Arancio, 20 Lake Ickuel, 21 Ain Draham. Circles and triangles represent areas with less and more than five specimens, respectively

Table 1 Number of examined specimens (*n*), mean PLSDA component 1 values Comp, cluster membership in ANOVA Tukey's-b post hoc (Tukey), latitude (Lt), longitude (Lg)

Code	Area	n	Comp1	Tukey	Lt	Lg
1	Esperia	6	1.27	5, 6, 7	41.39	13.69
2	Ischia	9	0.80	5	40.73	13.90
3	Napoli	2	1.76	-	40.86	14.19
4	Capri	9	-0.15	4	40.55	14.23
5	Punta Campanella	8	1.50	6, 7	40.58	14.34
6	Ottati	5	1.32	5, 6, 7	40.47	15.31
7	Castrovillari	1	1.69	-	39.82	16.22
8	Cosenza	12	1.61	7, 8	39.30	16.26
9	Sila Grande	2	1.33	-	39.28	16.50
10	Isola Capo Rizzuto	1	1.33	-	38.96	17.09
11	Monte Limina	8	1.05	5, 6, 7	38.41	16.18
12	Gambarie	12	0.84	5, 6	38.15	15.81
13	Portella Rizzo	7	-1.49	2, 3	38.21	15.5
14	Santa Lucia sul Mela	9	-1.58	1, 2, 3	38.14	15.28
15	Lipari	7	-1.26	3	38.49	14.93
16	Galati Mamertino	5	-2.17	1, 2	38.02	14.76
17	Madonie	7	-1.46	2, 3	37.90	14.00
18	Ficuzza	8	-1.58	1, 2, 3	37.88	13.38
19	Lago Arancio	4	-1.80	-	37.63	13.05
20	Lake Ickuel	13	-2.19	1	37.22	9.77
21	Ain Draham	2	-2.48	-	36.78	8.70
22	Balkans	13	2.13	8	-	-

outline of the tegumen and brachium and four on the valve that could be precisely identified, were considered as landmarks (type II and type III landmarks, Bookstein 1997), whereas the other points (sliding semi-landmarks) were allowed to slide along the outline trajectory to reduce uninformative variation (Dapporto et al. 2009). Compared to Dapporto et al. (2009) we halved the number of sliding semi-landmarks in order to reduce the variables (PCs) extracted by the following statistical analyses. Digital data for landmarks on genital photographs were carried out by using TPSDIG 2.16 and for the definition of sliders we used TPSUTIL 1.46.

Generalized procrustes analysis (GPA) was applied to the landmark data in order to remove non-shape variation in location, scale and orientation, and to superimpose the objects in a common coordinate system (Bookstein 1997). By using shape residuals from GPA, we calculated the partial warps containing shape information. Through the application of principal components analyses (PCA) to partial warps, we obtained relative warps (PCs) that can be used as variables in the PLS discriminant analysis. Moreover, PCs can be visualised by TPS deformation grids, which allows a visual comparison of shape differences. Partial and relative GPA, warp calculations and TPS visualisation were carried out by using TPSRELW 1.49.

The discriminant analysis proved to be a suitable method with which to clearly highlight the best combination of PCs

allowing distinctions among specimens belonging to different morphotypes (see Dapporto 2010a; Dincă et al. 2011 and literature therein). Indeed, PCs involved in the distinction among morphotypes often—but not always—explain the largest variance (Dapporto 2010a; Dincă et al., 2011). However, the use of discriminant analysis has often been criticised because of its strict assumptions (i.e. the homogeneity of variances among groups, the considerably lower number of predictor variables than cases, the non-multicollinearity of predictors; Mitteroecker and Bookstein 2011). Therefore, we carried out a partial least square discriminant analysis (PLSDA), which is unaffected by the sample characteristics that are common in morphometrics data. We used PC scores as predictors, and the 16 areas where at least five specimens were collected as grouping variables. The model obtained was then applied to areas comprising less than five specimens in order to predict the component scores of these cases. As revealed by previous studies on Satyrinae, including *M. jurtina*, the discriminant function 1 usually captures genitalia variations explaining differences related to morphotype membership, and proved a reliable method with which to discriminate between *M. j. janira* and *M. j. jurtina*. Moreover, graded variations in discriminant function 1 from the two extreme values capture the degree of hybridisation of the various populations (compare results of Dapporto et al. 2009 with Thomson 2011). We used

the mean values of the first PLSDA component from each of the 21 areas to model a high resolution distribution of the morphotypes of *M. jurtina*.

The availability of data from a large number of locations allowed a finer-scale comparison and a spatial modelling that had been impossible in previous studies (Dapporto et al. 2009). As a first step, we performed an ANOVA in order to determine whether the 16 areas, where at least five specimens were analysed, showed different values of the PLSDA component 1. We also applied Tukey's-b post hoc test in order to search for homogeneous subsets of areas.

GIS-based spatial analyses

Thereafter, an approach based on the geographic information system (GIS) was carried out to evaluate variation in genitalia shape over the study area. As a first step, we interpolated the observed mean PLSDA component 1 values belonging to the 21 sampling sites over the entire study region. There are several interpolation methods and their choice may greatly affect the quality of the result (Chaplot et al. 2006, and literature therein). Under the conditions of a strong spatial structure and anisotropy, the inverse distance weighting (IDW) generally performs better than the other methods (Chaplot et al. 2006). Since west Mediterranean butterflies (including *M. jurtina*) show abrupt variations in shape around particular sea straits (Dapporto et al. 2009; Dapporto 2010a), we used this method to predict genitalia shape over the study area. To highlight areas of particularly strong variation we also calculated and visualised the slope of the IDW interpolation.

Isobaths for the islands of Ischia and Capri were also reconstructed through IDW interpolation by using depth data belonging to nautical maps (1:30.000 Istituto Idrografico della Marina Italiana; <http://www.nautica.it/iim/>). Afterwards, on the basis of the reconstruction of the sea level eustatic variations in Italy given by Lambeck et al. (2004), we drew the presumed shorelines at different times, in order to infer the variation in island isolation from the end of the last glacial maxima up to recent times.

ANOVA was performed by using SPSS 15.0, PLSDA by using the MixOmics package of R and the GIS analyses by using the ArcGIS software with the Spatial Analyst extension.

Results

We obtained 20, 12 and 44 relative warps from the analyses of the tegumen, brachium and valva, respectively. The first component of PLSDA is highly correlated to valva PC1 (explained shape variance=53.69%, loading in PLSDA

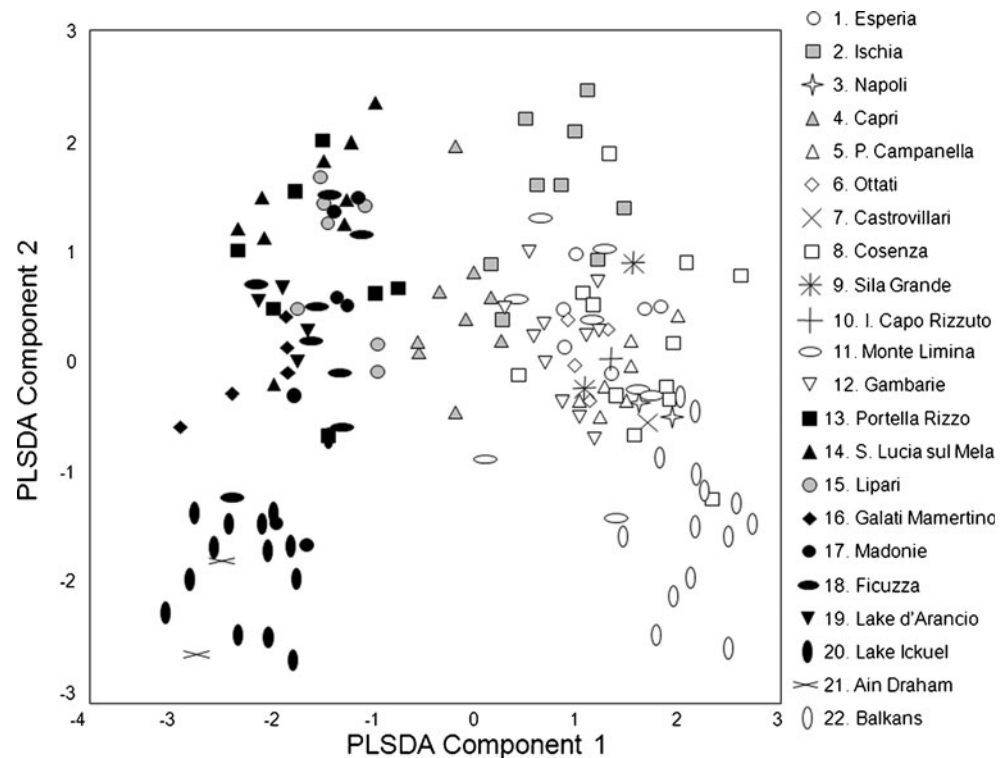
comp. 1=-0.570), brachium PC1 (explained shape variance=50.64%, loading in PLSDA comp. 1=0.518) and at a lower extent to tegumen PC2 (explained shape variance=28.69%, loading in PLSDA comp. 1=-0.371). The second component showed lower loading values, with PCs explaining much lower shape variance: tegumen PC6 (explained shape variance=2.28%, loading in PLSDA comp. 2=0.303), valva PC8 (explained shape variance=1.31%, loading in PLSDA comp. 2=-0.294) and brachium PC6 (explained shape variance=1.15%, loading in PLSDA comp. 2=0.282). The first component separated the specimens belonging to the Balkans and the Italian Peninsula from those belonging to Sicily and North Africa. Specimens from Capri and, to a lesser extent, from Gambarie, Monte Limina and Ischia were placed in an intermediate position. Component 2 separated Tunisia and Balkan specimens from those belonging to Italy (Fig. 2). Component 1 was highly related to the PCs explaining most of the shape variance (valva PC1 and brachia PC1), and we assume that it explained most of the overall variance in the sample. Actually, examination of the valvae and brachia deformations along these PCs largely agreed with previous descriptions of morphological dissimilarities between *M. j. janira* and *M. j. jurtina* (Fig. 3a).

ANOVA revealed strong differences in the values of component 1 in the study area ($n=137$, $F=114.133$, $P<0.001$). Tukey's-b post hoc test identified eight homogeneous subsets of areas. All areas from Sicily, Lipari and Tunisia were grouped in the first three clusters where no other areas were included (Table 1), thus revealing a high homogeneity between the specimens from these regions. The Italian Peninsula, Capri, Ischia and Balkans showed a different trend. Indeed, there was a trend in membership from group 8 to group 4 underlying a trend in component 1 values. The Balkans were restricted to group 8 on one side of the cline and Capri to group 4 (for details see Table 1).

The IDW interpolation of component 1 values indeed confirmed that variation along Tunisia and Sicily is very low, being much higher on the Strait of Messina, between the island of Capri and Punta Campanella and along the mainland area of Calabria (Fig. 3a). Such a pattern was described even better by the slope analysis, which showed an abrupt change in genitalia shape between Portella Rizzo and Gambarie and between Capri and Punta Campanella, and a medium steep area in Calabria and between Ischia and Napoli (Fig. 3b).

The reconstruction of ancient sea-levels based on the recent isobaths showed that Capri island was connected to the mainland during glacial maxima but remained isolated between 14 and 12 ka BP (Fig. 4a), whilst Ischia was separated only recently from the Italian mainland by two sea straits. According to isobaths and sea levels reconstruction, the sea strait between the

Fig. 2 Partial least squares discriminant analysis (PLSDA) displaying the relative positions of specimens belonging to the 21 areas. Only the areas with more than five specimens were used to construct the model. Components 1 and 2 are represented on the x- and y-axis, respectively



islands of Ischia and Procida were restored about 9 ka BP, while the strait between Procida and Pozzuoli appeared about 7 ka BP (Fig. 4b).

Discussion

Our data confirm that two morphotypes of *Maniola jurtina* occur in the study area. The differences in shape highlighted by geometric morphometrics largely reflect the descriptions of the two main populations inhabiting Europe (compare Fig. 3 and Thomson 2011). The western population (*M. j. jurtina*) is recognised as having a larger distal portion of the valva and a narrower dorsal process, while the eastern population (*M. j. janira*) shows a narrower distal part of the valva and a larger dorsal process). The distal parts of *M. j. jurtina*'s brachia are larger than those of *M. j. janira*'s (Dapporto et al. 2009; Thomson 2011). We also confirmed that only *M. j. jurtina* occurs in Tunisia and Sicily and shows minor variations along this area. Conversely, abrupt changes among populations occur in three areas: at the Strait of Messina (3 km) between Sicily and Calabria, between Capri and Punta Campanella (about 5 km), and between Ischia and the Italian mainland (about 2+2 km, Fig. 4). Indeed, the individuals of Punta Campanella and the neighbouring mainland specimens (Esperia, Ottati, Cosenza) show a similar genitalia shape, while the individuals collected in the immediate proximity of Sicily (Gambarie and Monte Limina) show transitional characteristics that

make them similar to the specimens collected in the islands of Ischia and Capri.

Comparison of available morphometric and genetic data

It is important to note that our work examined only genitalia shape. As a first step we discuss the available genetic data for this species and the reliability of the use of genitalia morphology to infer biogeographic patterns. A comprehensive analysis of mitochondrial and nuclear DNA has never been carried out on the Mediterranean populations of *M. jurtina*. However, comparison of allozyme variation and male genitalia morphology of *M. jurtina* over Western and Eastern Europe, as well as in some major islands, revealed a high correlation between these markers, which differentiate an eastern group inhabiting eastern Europe, Crete and northern Italy from a western group inhabiting Iberia, North Africa, and the islands of Sicily, Malta, Capri and Sardinia; Elba and Corsica islands were found to be inhabited by a hybrid population (Thomson 1987, 2011). This pattern is highly concordant with recent morphological studies (Dapporto et al. 2009; present study). Analyses of new allozyme loci (i.e. locus PhePro) allowed the recognition of a third central group of populations belonging to North Africa, Sicily and the Italian mainland (Habel et al. 2009). This result contrasts with both our own and previous results (Thomson 1987, 2011), as the Italian and North African populations share the same PhePro alleles but belong to two different morphological subspecies (Dapporto et al. 2009). The discordance of allozymes compared to concordant

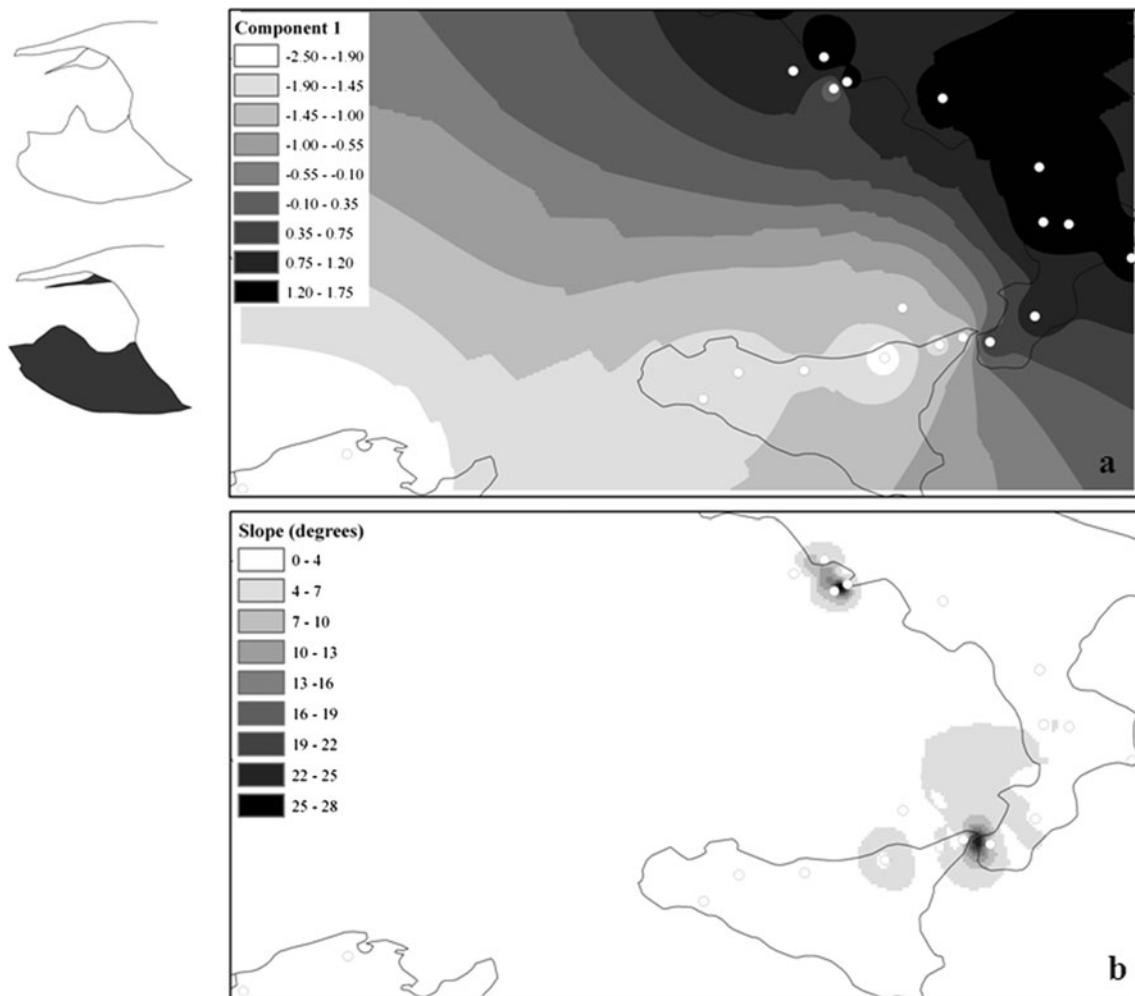


Fig. 3 Interpolation by inverse distance weighting (IDW) of component 1 values over the study area (**a**). The shapes of valvae and brachia corresponding to the maximum and minimum values of valva PC1 and brachium PC1 are shown beside the legend. Slope of the interpolated

values (**b**) showing maximum values on the Sicily-Calabria and the Capri-Sorrento sea straits and moderate values on the Ischia-Napoli sea strait and along Calabria

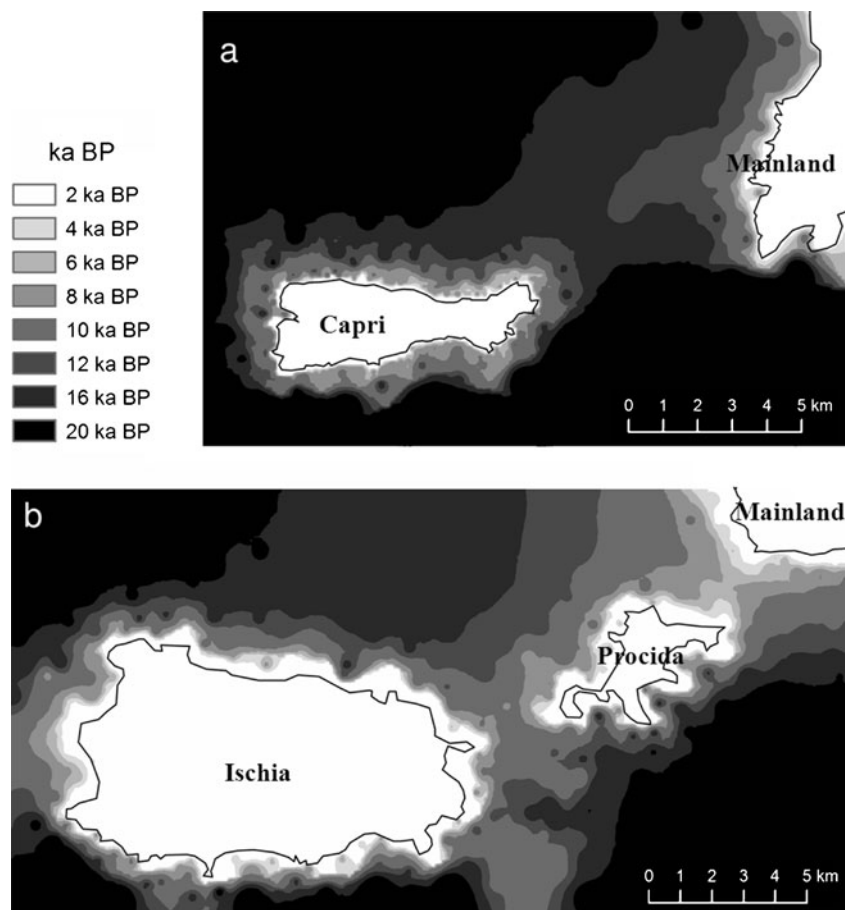
data on mtDNA and genitalia morphology between Italy and North Africa has also been observed in other species. *Melanargia galathea* and *Polyommatus icarus* show the same allozyme set in Italy and Tunisia (Habel et al. 2008, 2010), although in Tunisia two other distinct species (*Melanargia lucasi* and *Polyommatus celina*) are recognized on the basis of mitochondrial and nuclear DNA sequences as well as on the basis of morphological traits (Verity 1940–1953; Nazari et al. 2010; Wiemers et al. 2010; Dincă et al. 2011). From this perspective, the choice of the allozyme loci seems to influence the results strongly, since some alleles can be shared by highly differentiated taxa.

We also postulated that intermediate individuals from the mainland and islands might represent hybrid populations between the two morphotypes. There is extensive evidence of the existence of hybrid populations of *M. jurtina* showing intermediate phenotypes in eastern France (Thomson 1987, 2011; Schmitt et al. 2005; Dapporto et al. 2009). Diverging

populations are often found on islands due to genetic drift, which may be responsible for the observed island-mainland differences; in this case, however, the variation should be expected to differ stochastically among islands. On the contrary, island populations from the majority of circum Italian islands (Corsica, Elba, Giglio, Pianosa, Ischia and Capri) show a similar intermediate shape between the Italian and the Spanish/North African morphotypes that is also found in the French suture zone (Dapporto et al. 2009; Dapporto 2010; Thomson 2011). From this perspective, the most parsimonious hypothesis is that the intermediate populations of Ischia, Capri and Calabria represent hybrids between the two subspecies.

Until a comprehensive assessment of mitochondrial and nuclear loci is carried out, we conclude that male genitalia are most likely a suitable marker for both phylogeography and the detection of hybrid populations of *Maniola jurtina*. However, future studies are required to definitively assess

Fig. 4 Reconstruction of isochronous shorelines for Capri (a) and Ischia (b) at different times by coupling present-day isobaths and past sea-level reconstructions



the relationships between DNA sequences, allozymes variation and morphological traits.

What about the observed distribution?

The hypotheses reconstructing the post glacial colonization routes of European/Mediterranean species are dominated by the assumption of a contraction toward southern areas (Iberia, Italian Peninsula, Balkans, North Africa) during glacial periods followed by rapid northward expansions at the beginning of the warm phases (de Lattin, 1967; Taberlet et al. 1998; Hewitt 1999; 2000; Schmitt 2007). There are many examples of organisms matching this hypothesis, including several butterflies (Porter et al. 1997, Habel et al. 2005; Schmitt 2007; Dapporto 2010b); this scenario suggests that such a generalized hypothesis is probably responsible for most of the biogeographic patterns of European species. A crucial point is whether suture zones tend to remain geographically stable in time (Hewitt 1996, 1999, 2000).

Therefore, according to a first hypothesis, *M. j. janira* expanded from the Balkans and Italy while *M. j. jurtina* expanded from North Africa and Spain until they met in the large suture zone between France and the British Isles (Schmitt et al. 2005b; Thomson 2011). The existence of a narrow hybrid

zone in Calabria, which does not occur in Sicily, suggests that the dispersal of the two morphotypes across the Strait of Messina could have involved the movement of *M. j. jurtina* from Sicily to Calabria and not vice versa. According to an even more unlikely assumption, *M. j. jurtina* might have dispersed not only to Lipari, but also to Capri and Ischia, hundreds of kilometres over the Tyrrhenian sea. Such a long dispersal might have originated hybrid populations on these islands and not on the neighbouring mainland areas, which show no evidence of intermediate populations.

The alternative hypothesis assumes that *M. j. janira* has replaced the ancestral *M. j. jurtina* on the Italian Peninsula by introgressive hybridisation (Mallet, 2005; Dapporto et al. 2009, 2010a). Such an invasion could have been filtered by the short sea channels separating the populations of Ischia and Capri. There is large evidence that, besides some macro-refugia (e.g. the three peninsulas and the Maghreb), several reduced areas may function as independent micro- and crypto-refugia (reviewed by Provan and Bennett 2008; Ashcroft 2010); islands, in particular, have been shown to function as very efficient refugia (Masini et al. 2008; Médail and Diadema 2009; Dapporto 2010a; Dapporto et al. 2009, 2011b). In this regard, butterflies present on islands might be ancient relicts surviving from multiple expansion/contraction events. This

process is likely to have occurred in the very isolated island of Sardinia, which has never come into contact with the nearest Italian mainland. On the contrary, all the islands analysed in the present work experienced territorial contiguity or a very high proximity with the Italian mainland during the last glacial maximum. The occurrence of these territorial connections has forcedly smoothed any possible ancestral island-mainland diversification, which makes any hypothesis supposing the persistence of earlier ancestral populations meaningless (Dapporto et al. 2009; Dapporto 2010a).

Our data also suggest that we are observing an ongoing dynamic process. Ischia, which is isolated from the mainland by two recently established (9–7 ka BP) sea straits of about 2 km, turned out to be more introgressed than Capri, which is separated from the mainland by a single sea strait more than 5 km long (Fig. 4). Moreover, there is evidence that the range expansion of *M. j. janira* is still operating at the tip of Calabria, where the replacement is incomplete. Our alternative model minimises the putative movements of the morphotypes through sea channels, and may also explain the occurrence of the observed asymmetrical area of hybridisation between Sicily and Calabria.

The proposed pattern contrasts with the hypothesis of the stableness of suture zones. Recent reviews suggest that macro-ecological patterns are not stable in time (Ricklefs 2008; Fisher et al. 2010). The abundance and the distribution of single species are highly positively correlated, but they can also vary greatly over time (Blackburn et al. 1998). Differential variations at distributional edges, in both abundance and distribution, may allow the ingression of a positively varying lineage into the area occupied by the negatively varying lineage. The east–west spreading movement of the *M. j. janira* propagule is not surprising since, in a recent work on the Mediterranean areas, Blondel et al. (2010) noted that “[...] the Irano-Turanian region has contributed many more taxa to the Mediterranean region than the opposite [...]”. The same authors also suggested that the habitat degradation and simplification produced by humans can favour the spread of the “oriental” elements of flora and fauna evolved in particular dry and climatically extreme habitats. Rapid range expansions in butterflies is widespread since 63% of 35 non-migratory European species (comprising *Maniola jurtina*) have shifted their ranges to the north during this century, probably as a response to global warming (Parmesan et al. 1999).

Timing of the supposed invasion

Our hypothesis postulates that the expansion of the eastern morphotype of *M. jurtina* toward the Italian islands has been impeded by sea straits, and assumes that the islands were already separated from the mainland when the invasion from Balkans took place. Several Italian offshore islands, connected to the Italian mainland during glacial maxima, became

isolated during the interglacial phase. This process can be roughly reconstructed by comparing recent bathymetry and sea-level changes. Our reconstruction shows that Capri was already isolated from Punta Campanella about 12 ka BP. Regarding Ischia, the reconstruction is more complex. Indeed, the two sea channels separating this island from the mainland were restored at different times because of their different depth (7 and 9 ka BP, Fig. 4b). However, these data are based on the reconstruction of sea-level variations, regardless of possible vertical tectonic movements (Lambeck et al. 2004). The area between Ischia and Napoli is well known for its tectonic activity, which produced significant and cyclic uplifts that could have kept up with the effects of sea level changes (Lambeck et al. 2004, and references therein). In this respect, the landbridge that connected Ischia to the mainland could have remained above sea level for a longer or shorter time than can be expected by simple examination of sea-level variations. The expansion of the eastern morphotype into the Italian Peninsula is more than likely to have occurred later than 7 ka BP, a relatively recent time compared to the onset of deglaciation that occurred 18 ka BP.

We provide empirical support for this hypothesis by suggesting a recent replacement of *M. j. jurtina* by *M. j. janira* along the Italian peninsula that could be still operating in Calabria, Ischia and Capri. Species showing a significant range expansion during the last 100 years moved across a distance of 35–240 km (Parmesan et al. 1999). If we consider that the distance between the Strait of Messina and the Alps ridge separating Italy from the Balkans is about 1,200 km, it might have taken from 3,400 to 500 years for the eastern population to spread over the Italian mainland.

Conclusion

Our data suggest that ongoing climate change may drastically affect the distribution of genetic lineages of Lepidoptera, and has a high potential to modify the current European biogeographic pattern. The results obtained do not apply to a single species: as a matter of fact, it has been demonstrated recently that many butterflies show different morphotypes in Italy compared to most West Mediterranean mainland and islands (Cesaroni et al. 1994; Dapporto, 2010a; Dapporto et al. 2011a b; Dincă et al. 2011). This suggests that the colonisation route hypothesised in the present work may be a widespread pattern in butterflies, which can probably be applied to several distributions of invertebrates and vertebrates (e.g. Fattorini 2009). Some populations of amphibians, reptiles and mammals from the Italian islands are genetically very similar to those occurring in North Africa, and highly differentiated from those occurring in the Italian mainland (Podnar et al. 2005 for *Podarcis* lizard; Giovannotti et al. 2007 for *Chalcides* skink; Stöck et al. 2008 for *Bufo* green toad; Biollaz

et al. 2010 for *Myotis* bat). As with butterflies, cases of incongruence between islands and their neighbouring mainland could be explained by a rapid invasion of the mainland by lineages belonging to neighbouring glacial refugia with a relatively high inertia on islands.

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