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Twenty Years of Molecular Biogeography in the West Mediterranean Islands of Corsica and Sardinia: Lessons Learnt and Future Prospects

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1. Introduction

The Mediterranean Sea comprises a wide array of insular systems. Sardinia and Corsica are respectively the second and forth-largest islands of the Mediterranean Sea and they are environmentally complex due to their topography and orography. Owing to their central position in the Tyrrhenian Sea (Figure 1) humans started settling on the islands relatively early, during the Mesolithic. Pliny and Ptolemy were among the first to briefly mention the islands' fauna. More systematic surveys of their biodiversity started around 300 years ago, when Sardinia became part of the Kingdom of Sardinia ruled by the House of Savoy and Corsica was incorporated into France [1]. Our current knowledge of the islands' biological diversity can be considered quite accurate; the fauna is relatively species-poor compared to the surrounding continental areas, still rate of endemism is high, approaching about 7% for Sardinia [2]. Most of the Corsican-Sardinian endemisms show clear affinity with species distributed across that part of Southern Europe that embraces Northern Spain and Southern France. Some of these elements are also closely related to species occurring in Central insular and peninsular Italy (Tuscan Archipelago and coastal areas of Tuscany; Figure 1). These concordant, yet disjunct, distributions (peri-Tyrrhenian hereinto) are shared among a variety of unrelated organisms, from plants to invertebrates and vertebrates (see [3] for a synthesis) all having very low (if any) potential for long distance, over-sea dispersal.

Recurrent patterns in geographical ranges of unrelated species have traditionally attracted the interest of biogeographers because they can be reasonably related to the same underlying event(s). In the case of the Corsica-Sardinia system, the presence of a pre-Miocene land bridge connecting the different landmasses had been initially hypothesized [4]; affinities of

nowadays allopatrically distributed lineages were consequently interpreted under a dispersal scenario. The advent of the theory of plate tectonics allowed a detailed reconstruction of the geological history of the islands [5,6] (see Figure 1 and next chapter for details) and induced many authors to favour vicariance over dispersal as the main process that originated the islands' biodiversity [1,3].

The uniqueness of a biogeographic situation with several co-distributed, yet unrelated, species all presumably sharing the same history did not escape the attention of molecular evolutionary biologists. The Corsican-Sardinian system offers the opportunity to test explicit biogeographic hypotheses in light of a well-known geological background; the available geological time estimates can be used to test for the clocklike nature of genetic divergence and eventually calibrate rates of molecular evolution. In 1990 the first molecular data ever on a Corsican-Sardinian endemism with Iberian affinities (newts of the genus *Euproctus*) were included as part of a review on molecular island biogeography [7]. Since then a good wealth of molecular work has been done on a variety of terrestrial and freshwater species (both invertebrates and vertebrates) based on different molecular markers (Table 1). The molecular and analytical tools employed in those studies reflect the unparalleled technological and analytical development that the field has witnessed in the last two decades.

In light of the central importance that insular settings have had in the development of the evolutionary thinking, we assembled this review with the aim to specifically address the following points. First, we will present a synthesis of the most representative molecular studies (i.e. explicitly centred on Corsica-Sardinian endemisms and not part of larger phylogenetic studies) conducted on animal groups whose distribution is limited to the Corsica-Sardinia system and surrounding continental landmasses involved in the past geological evolution of the islands. Second, we want to test for each of these groups whether phylogenetic relationships fit those expected if cladogenetic events were due to vicariance only. Third, we will summarize the available molecular estimates of divergence times to discuss how they relate to current views on the geological evolution of the landmasses. Fourth, we will summarize whether for each group substitution rates accumulate linearly over time or not (if this was tested in original study) and compare rates based on the same markers and calibrated using the very same geological event(s) across taxonomically unrelated groups. Finally, to place this review in a larger context and to ultimately suggest future avenues in the study of the evolution of insular biota we will explore how molecular evidence on the Corsica-Sardinia system relates to comparative phylogeographies available for other insular systems.

2. Geographical setting

Corsica is located in the Tyrrhenian Sea south of France, west of Italy and north of the island of Sardinia. Its surface totals about 8700 km² extending for 183 km; the island is 83 km wide and it is about 90 km away from Italy (Tuscany), 170 km from Southern France and it is separated from Sardinia by the Strait of Bonifacio (minimum width 11 km). Mountains comprise about two-third of the island forming a single chain that runs in a north-south direction.

Sardinia, with an area of about 24000 km², is the second largest island of the Mediterranean Sea. The island is 270 km long and 145 km wide and is almost equally distant from peninsular Italy on the east (187 km) and North Africa (Tunisia) on the south (184 km). Many small islands and islets surround Sardinia, the largest being the island of Sant' Antioco (109 km²) situated at its southwest tip. Most of its territory is mountainous (about 80%) and a number of mountain chains can be identified separated by intervening alluvial plains and flatlands, the largest being the Campidano plain in the southwest part of the island.

3. Geological history

Corsica and Sardinia are old continental islands and their geological evolution has been reconstructed in good details. A consensus on the overall process of formation of the two islands exists. Some questions are still open, though, regarding the timing of final detachment of two islands from the continent and pattern and timing of contacts between them. Traditionally, the split of Corsica and Sardinia from the Iberian Peninsula as a single microplate had been dated at about 29 million years (Myrs) ago; the rotation of the microplate and the disjunction of the two islands started 15 Myrs ago and was completed by 9 Myrs ago [5,6,8,9,10,11,12]. Recently, new geological data challenged this scenario. According to [13,14] the beginning of the split of the microplate should be dated at 24-20 Myrs. The maps presented in [15] support these views but also suggest that the microplate remained connected to the southern edge of Palaeo-Europe during its anti-clock wise rotation through a land bridge that will constitute the future Maritime Alps and the Ligurian Apennines (Italy). The final detachment of the microplate from the continent was contemporary with the onset of the uplift of Tuscany in continental Italy and occurred in the Pliocene (around 5 Myrs ago). The interaction between the Corsica-Sardinia microplate and the Apennines, which were then being formed, caused the emergence of the Tuscan Archipelago, including the islands that later became incorporated in the mainland (the so-called "fossil islands") [11]. Further connections between the Corsica-Sardinia microplate and the continent were probably established during the Messinian Salinity Crisis (5.7-5.3 Myrs) [10,12]. Finally, sea-level oscillations, which occurred repeatedly in the Quaternary at each ice age (from 2 to 0.5 Myr ago), led to connect northern Corsica to Tuscany and southern Corsica to northern Sardinia [16-19]. It is worth noting that all the connections of the two islands to the continent after the initial detachment of the microplate were short-lived, regardless of how many times they happened and when [20,21]. A schematic representation of the alternative views on the geological evolution of the area is given in Figure 1.

In spite of the temporal vagaries outlined in the previous paragraph, the geological cladogram of the area, that is the representation of relationships among areas based on their geological history, can be summarized as follows. The Iberian Peninsula is basal in the cladogram and the two islands are each other's sister areas. When also Balearic Islands, Tuscan Archipelago and continental Italy are considered, then Balearic Islands would be sister to the Iberian Peninsula while Corsica and Sardinia would no longer be sister areas but Sardinia would be basal to a clade formed by Corsica, Tuscan Archipelago and coastal areas of Central Italy. The two geological area cladograms are depicted in Figure 2.

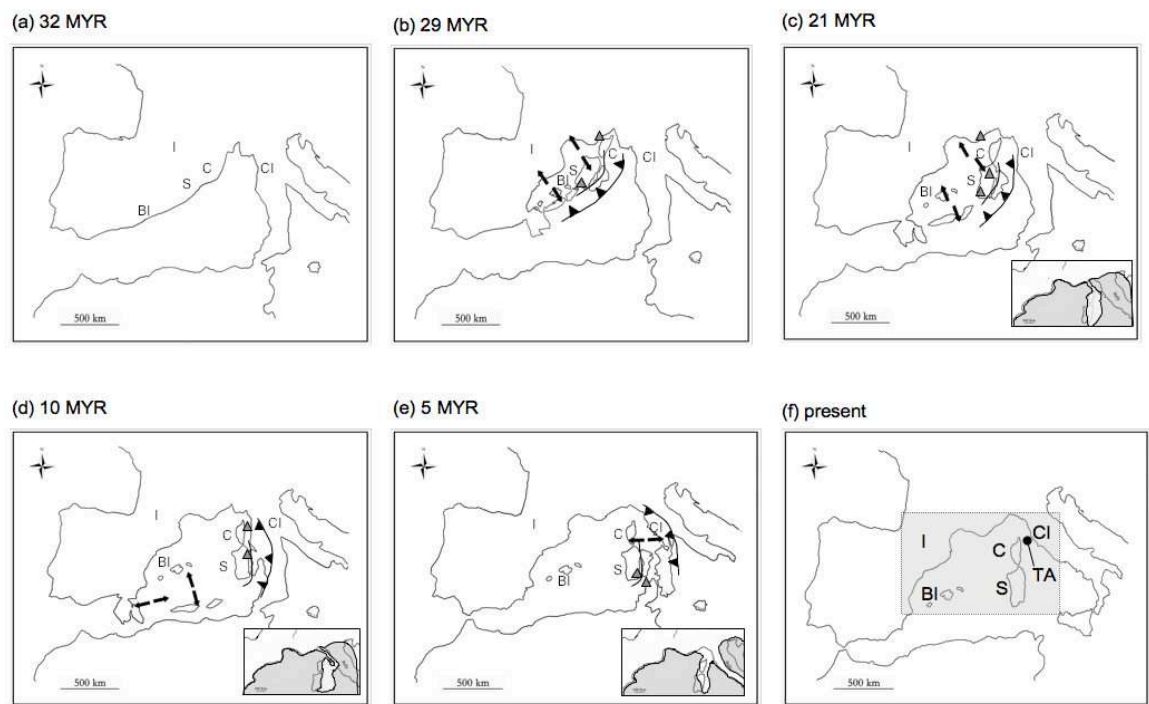


Figure 1. Geological evolution of the peri-Tyrrhenian area. Panels (a) to (e) show reconstructions of the split of the Corsica-Sardinia microplate from the Iberian Peninsula, its subsequent rotation and interaction with the still extant Tuscan Archipelago and the current coastal area of Tuscany (fossil islands; see text). Approximate age of each geological phase is also given. Bottom right inlets in panels (c), (d), and (e) show the interactions between Corsica-Sardinia and Continental Italy between 21 and 5 Myr proposed by [15] as alternative to the classical scenarios shown in the larger panels (grey and white shaded areas correspond to sea and land, respectively). Black and grey triangles indicate oceanic subduction and thrusting. Panel (f) shows the present geographic location of main areas considered in the study: the Iberian Peninsula (I), Balearic Islands (BI), Sardinia (S), Corsica (C), Tuscan Archipelago (TA) and Continental Italy (CI). Maps were drawn on the basis of present geography.



Figure 2. Geological area cladograms of the peri-Tyrrhenian area. The cladogram on the left depicts relationships only when the three major landmasses are considered while on the right are expected relationships when additional areas are also included (see text for details).

4. Ecology and endemism

Corsica and Sardinia have a Mediterranean climate characterized by hot and dry summers and mild and wet winters. Rainfalls are concentrated in autumn and winter with sporadic showers in spring. Owing to the presence of numerous mountains, the Mediterranean climate of the coastal zone (between the sea level and 600 m of altitude) changes into a milder, cooler and wetter climate in the temperate mountain zone comprised between 600 and 1800 m above the sea level. In Corsica, where elevation reaches 2700 m of altitude, it is possible to identify a high alpine zone (between 1800 and 2700 m) where snow-caps and small glaciers are not infrequent.

The vegetation of the islands reflects the climate altitudinal zones. In the coastal areas Mediterranean forests, woodlands, and shrubs predominate with evergreen sclerophylls. Much of the coastal lowlands have been cleared for agriculture, grazing and logging, activities that have considerably reduced the forest cover. Maritime Pines interspersed with forests of deciduous trees are typical of middle elevations. Above 1800 m of altitude (Corsica only), sub alpine shrub lands progressively substitute forests of Corsican Pine, Silver Fir and European Beech. Endemic plant species are chiefly restricted to high altitudes in Corsica and to coastal areas in Sardinia [3].

Corsica and Sardinia are faunistically impoverished as compared to potential surrounding continental sources. Based on taxonomic and faunistic considerations a three-phase model of colonization of the islands (pre-Miocene, Messinian and Quaternary; a fourth phase considers species introduced by humans) has been proposed [3]. The first phase would correspond to the detachment of the microplate from the Iberian Peninsula. Most of the endemic species that are nowadays distributed in Sardinia and Corsica (either in common between the two islands or unique to each of them) have differentiated from ancestors that were supposedly co-distributed on the microplate and the Iberian Peninsula when these were still forming a single landmass. Thus, the origin of these lineages is at least 29 Myrs old, even though we cannot exclude that cladogenesis predated geological splits. Invertebrates are particularly well represented. Freshwater planarians of the genus *Dugesia* belong to this stock along with multiple endemic lineages of terrestrial gastropods (genera *Rupestrella*, *Solatopupa*, *Hypnophila* and *Tacheocampylaea*). Similarly, examples are found among earthworms (genus *Hormogaster*), crustaceans isopods (both epigean and hypogean, aquatic and terrestrial with the genera *Proasellus*, *Stenasellus*, *Helleria*, *Nesiotoniscus*, *Tiroloscia* and *Lucasius*), arachnids (harvestmen of the genera *Parasiro* and *Scotolemon*, the Acari genera *Damaeus* and *Oribatella* and at least five lineages of pseudoscorpions) and centipedes. Insects are present with different orders. Among others, stoneflies and beetles are very interesting biogeographically. The stonefly genus *Tyrrhenoleuctra* includes three endemic lineages; cave Bathysciine beetles are rich in endemism with at least 11 species (genera *Ovobathysciola*, *Patriziella* and *Speonomus*) likewise are scarab beetles with the genera *Elaphocerida*, *Triodonta*, *Cetonia*, *Thorectes* and *Typhoeus*. Amphibians and Reptiles also contributed to this early phase of colonization. Urodela share no species with any of the adjacent continental landmasses and include at least six endemic species (two genera; newt *Euproctus* and salamander *Speleomantes*). The endemic lineages of the lizard genera *Archeolacerta*, *Algyroides* and *Podarcis* also belong to this early stock of colonizers. No mammalian representatives of this ancient stock are still extant; known from fossil records are the perissodactyls *Atalodon* and *Lophiodon* and the ruminant *Amphytragulus boulengeri*.

During the Messinian Salinity Crisis (MSC; 5.7-5.3 Myrs) the Mediterranean Sea almost completely dried up. The MSC was short-lived; nonetheless it allowed a number of species to reach the islands. These constitute most of the extant Corsican-Sardinian fauna, although none of them had diversified on the islands into endemic species. The only endemic subspecies is the colubrid snake *Natrix natrix cettii*. Species that reached the islands during the MSC are typical of a warm to hot climate because they had to withstand the harsh conditions of the drained and hyper saline Mediterranean basin. Colonization proceeded along two major paths from south and east. Sardinia and Corsica thus share earthworms, arachnids, insects, reptiles and many fossil mammals with North Africa and Sicily. An eastern wave of colonization from continental Italy carried to the islands land snails, amphibians, reptiles and mammals (the last three groups left representatives almost exclusively in the fossil records).

The last connection(s) between our insular system and the adjacent continent (Central Italy) took place during the Quaternary ice ages. These connections were relatively short-lived, allowed dispersal of species adapted to a temperate to cold climate and have originated no extant endemism. Particular abundant is the mammalian fossil record, which includes extinct species of deer, wild boars, dwarf elephants, giant water voles and macaques. The endemic Sardinia pika (*Prolagus sardus*), a primitive lagomorph of Quaternary origin, went extinct in the late 1700s or early 1800s, probably due to a combination of habitat loss, predation and competition with introduced alien species [22].

Humans have started introducing species on the islands intentionally or accidentally since historical times. The extinction of much of the pre-Quaternary fauna is due to human activities (hunting above all), competition with alien introduced species or a combination of both. The Barbary partridge (*Alectoris barbara*) is an example of an introduced bird. Among mammals, rats, mice, hedgehogs, martens, weasels, wild cats and boars, follow deer, red deer and mouflons are all introduced. Some of them have been on the islands long enough to acquire unique morphological features that granted them a sub specific rank (the red deer *Cervus elaphus corsicanus* and the mouflon *Ovis orientalis musimon*).

5. The data set

Available molecular data on Corsica-Sardinia endemisms, on their continental Iberian counterparts (and/or insular and continental Central Italian when existing) are summarized in Table 1. They cover four classes of invertebrates and two classes of vertebrates. While a few studies employed simultaneously markers of different origin (mitochondrial and nuclear), the vast majority is based upon mitochondrial DNA (mtDNA) only; *Cytochrome Oxidase subunit I* (COI), the large (16S) and small (12S) ribosomal subunits are the most frequently used genes. Regardless of the type of marker used, number of lineages discovered molecularly exceeds those assumed on the basis of morphology alone (i.e. nominal taxa). Exceptions to this otherwise generalized pattern are cave beetles and newts but for both groups a one-species one-population sampling strategy was used [23-25]. In two circumstances (the terrestrial isopod *Helleria brevicornis* and the Bediagra rock lizard) the same lineage is distributed across

predicted phylogeographic breaks [26,27]. For *Helleria brevicornis* the same mtDNA haplotype has been found in Southern France, Central Italy and on three islands of the Tuscan Archipelago. One mtDNA lineage of the Bediagra rock lizard is in common between Sardinia and Corsica. Conversely, no haplotype sharing was detected for any of the other analyzed taxa.

Class	Taxa	N of nominal species	N of lineages ¹	Distribution (N of lineages) ²	Molecular marker	Molecular clock ³	Source
Oligochaeta	Earthworms <i>Hormogaster</i>	2	4	I (2)/S (1)/TA (1)	Allozymes (26 loci)	No	[35]
Gastropoda	Land snails <i>Solatopupa</i>	6	8	I (4)/S (1)/C (2)/TA (1)	mt- (12S, 16S, COI)/ nucDNA (H3)	Yes	[37,39]
Malacostraca	Aquatic cave Isopods <i>Stenasellus</i>	2	6	I (2)/S (2)/C (1)/ CI (1)	Allozymes (15 loci) mtDNA (COI)	Yes	[33,40,56]
	Terrestrial Isopods <i>Helleria</i>	1	6	I (1*)/S (3)/C (2)/TA (1*)/CI (1*) * same lineage	mtDNA (12S, 16S, COI)	Yes	[26]
Insecta	Stoneflies <i>Tyrrhenoleuctra</i>	3	5	I (2)/BI (1)/C (1)/ S (1)	Allozymes (11 loci) mtDNA (12S, COI)	Yes	[34,41]
	Cave beetles <i>Ovobathysciola</i> <i>Patriziella</i> <i>Anillochlamys</i> <i>Speonomus</i>	11	11	I (4)/ S (7)	mtDNA (COI)	Yes	[25]
Amphibia	European newts <i>Euproctus</i>	2	2	S (1)/ C (1)	mtDNA (12S, 16S, Cytb)	Yes	[23,24]
Reptilia	Bediagra rock lizard <i>Archaeolacerta</i>	1	2	S (1)/C (2*) *one lineage is shared with S	mtDNA (ND4, tRNA ^{SER, LEU, HIS})	Yes	[27]

¹ Following the criteria in [65]; ² I = Southern France + Iberian Peninsula; BI = Balearic Islands; S = Sardinia; C = Corsica; TA = Tuscan Archipelago (extant islands); CI = Continental Italy (mostly fossil islands; see Introduction and Figure 1); ³ Linearity of rates tested and/or calibration given in the study source.

Table 1. Summary of taxa with a Corsica- Sardinia- Iberian Peninsula distribution studied molecularly. For each group we give the nominal number of species, the number of lineages identified molecularly, the geographical distribution of such lineages, the markers employed, whether linearity of substitution rates has been tested and whether an explicit calibration of the molecular clock has been proposed in the original study.

When more than one conspecific population per geographical area was considered, molecules often revealed multiple lineages that are more closely related to one another than they are to any of those distributed on the other landmasses. This suggests that within-area diversification took place after the geological splits.

6. Hypothesis testing and TreeMap analysis

Was vicariance hence predominant over dispersal in promoting speciation in Corsica-Sardinia-Iberian taxa, as expected given the low dispersal capability of the groups listed in Table 1? If so, relationships within groups should mirror the geological area cladogram of the landmasses they occupy (see the Geological history section and Figure 1 for details). In other words, a vicariance scenario would be supported if the phylogeny of a given group were congruent with the known sequence of vicariant events as determined by geology [28]. To test this hypothesis, we used an approach initially developed to detect co-speciation in host-parasite systems and later on applied to biogeography [29,30]. It should be noted here that we had no access to raw datasets for any of the study cases based on allozymes included in this review. Furthermore, papers based on retrievable sequence data considered, with the sole exceptions of land snails and rock lizards, few populations and individuals (often just a single population per species). All this hampered applicability of the recently developed Approximate Bayesian Computation (ABC) approaches. ABC integrates the many parameters typical of any population genetics study into a Bayesian framework and takes advantage of the flexibility of the Bayesian statistics to derive inferences. ABC, however, arose primarily in the field of population genetics to investigate the demographic history of populations and implicitly assumes a dense sampling in terms of both individuals/populations and loci [31]. We hence limited ourselves to compare branching patterns of molecular phylogenies (as presented in the original papers; Table 1) to the area cladogram to reconstruct the alleged “host-parasite” associations (where the hosts are the geographic areas and the parasites are lineages of a given group). Associations between the molecular phylogenies and the area cladograms as well as all the subsequent statistical analyses were carried out in TreeMap 1.0 [32]. We used the heuristic option to reconcile the area and the group trees and to find a single optimal reconstruction. We tested the significance of the fit between the host and parasite trees by generating 10000 random “parasite” trees with the same number of taxa and “host-parasite” associations. We then measured how the random parasite trees fit the observed parasite trees in comparison with the area cladogram. The proportion of random gene trees that have the same (or greater) number of speciation-separation events as the observed tree is the probability of obtaining the observed value by chance alone. The null hypothesis is that the area cladogram and molecular phylogenetic trees are independent. TreeMap distinguishes and counts the following events: speciation by area, speciation within area, migratory and sorting events. The latter are assumed due to extinction and/or sampling errors; they hence reflect instances where “parasites” were expected to occur but do not.

Results of these analyses are summarized in Table 2. No migratory events were detected for any of the associations tested. The analysis was significant at the 0.05 level or below for the

subterranean aquatic isopod *Stenasellus* (allozymes and mtDNA), stoneflies (*Tyrrhenoleuctra*) and European newts (*Euproctus*). For these groups we have thus to reject the null hypothesis of no association between molecular relationships and the area cladogram. This implies that vicariance has been the main force driving their diversification.

Taxa	Events				P*
	Speciation by area	Speciation within an area	Migratory	Sorting	
Earthworms <i>Hormogaster</i>	3	2	0	1	0.071
Land snails <i>Solatopupa</i>	2	5	0	4	0.610
Aquatic cave Isopods <i>Stenasellus</i> (Allozymes)	4	2	0	1	<<0.001
Aquatic cave Isopods <i>Stenasellus</i> (mtDNA)	4	1	0	1	<0.001
Terrestrial Isopods <i>Helleria</i>	1	8	0	9	0.985
Stoneflies <i>Tyrrhenoleuctra</i>	4	1	0	0	<<0.001
Cave beetles <i>Ovobathysciola</i> <i>Patriziella Anillochlamys</i>	2	4	0	0	0.410
Cave beetles <i>Speonomus</i>	2	2	0	0	0.288
European newts <i>Euproctus</i>	2	0	0	0	<<0.001
Bediagra rock lizard <i>Archaeolacerta</i>	2	7	0	3	0.866

* based on 10.000 random permutations; P is significant when ≤ 0.05

Table 2. Summary of the reconstruction of lineage-area assemblage performed in TreeMap [32]. For each group TreeMap sorts the total number of scored events into four categories (columns 2-5; see text for details). The last column reports the significance of the observed fit between the area cladogram (see Figure 1) and the molecular phylogenies; the null hypothesis is that there is no association between them.

Aquatic isopods of the genus *Stenasellus* are highly adapted to subterranean life, they spend their whole life cycle in subsurface freshwaters and active dispersal can only happen when such a habitat is continuous [33]. Given these characteristics, it was not unexpected to find molecular relationships (regardless of the markers employed) to be remarkably in agreement with the palaeogeography of the area. Stoneflies are very poor fliers and spend most of their life cycle as nymphs in running freshwaters [34]. The terrestrial winged adults are short-lived and tend to stay close to the water edge to reproduce. Considerable oversea dispersal is difficult to hypothesize. Likewise unrealistic would be to invoke between islands sea dispersal for European newts, given the strict intolerance of amphibians to salt water [23,24].

TreeMap analyses were not significant for earthworms, land snails, terrestrial isopods, cave beetles and rock lizards, groups where the potential for dispersal is also low. For none of these

groups TreeMap suggested multiple colonization events of the islands. Genetic relationships in the earthworm genus *Hormogaster* do not match the area cladogram, because the lineage from the Elba Island is basal to the Sardinian ones [35], contrary to what expected on the basis of geological considerations alone (Figure 2). These results are likely due to the lack of resolution of the markers employed (allozymes) coupled with an incomplete taxon sampling [35]. Relationships within the genus and the family (Hormogastridae) are problematic and still in need of additional work based on as exhaustive taxon coverage as possible [36]. For land snails of the genus *Solatopupa* we found a relatively high number of within-area speciation and sorting events (Table 2). In particular, *S. guidoni* has diversified within the Sardinia-Corsica-Tuscan Archipelago area into mtDNA lineages that maintained a substantial morphological uniformity in shell and genital traits [37]. These insular lineages are not reciprocally monophyletic as expected if vicariance had been the only cause of divergence; haplogroups found on Sardinia and Elba Island are embedded within some of those distributed in Corsica. The sorting events detected are likely due to episodes of extinction because the species is nowadays absent from ecologically suitable areas where it has been reported in the past [37,38,39]. The high number of speciation within an area and sorting events as opposed to the very few speciation by area episodes justify the lack of fit between the phylogeographies of both the terrestrial isopod *Helleria brevicornis* and the Bediagra rock lizard and a purely vicariant model of divergence [26,27]. In either case, diversification started in the Pliocene, much later than the completion of the detachment of the Corsica-Sardinia microplate from the Iberian Peninsula. Mitochondrial DNA genealogies support relatively recent between-islands dispersal as demonstrated by the intermingling of haplotypes originating from the two islands. For the isopod, historic human-mediated transport has been also postulated [26]. None of these explanations applies to cave beetles, owing to their strict association with the subterranean environment [25]. In this case, the TreeMap analyses might have been partially distorted by the fact that the lineages considered in [25] are absent from Corsica (hence only two areas could be included in the analyses) and by the strong bias in number of species included in the study in favour of Sardinia. Consequently, speciation-by-area events are either the same or half of those detected within areas.

7. Divergence times and molecular rates

The peri-Tyrrhenian area offers at least two independent geological time estimates for calibrating rates of gene evolution (the split of the microplate from the Iberian Peninsula and the split between the two islands) within the same geographical setting across a variety of unrelated taxonomic groups. All the studies centred on this system but one took indeed advantage of this opportunity (Table 1). With the sole exceptions of [40,41], where calibration of sets of allozymic loci was attempted, all other studies exclusively considered mtDNA. Table 3 summarizes the main results. Linearity (i.e. acceptance of the molecular clock hypothesis) of rates was rejected on the whole for stoneflies only [34], while at least some (if not all) of the gene partitions tested in the other studies passed the molecular clock test. A remarkable slowdown in rates was detected for the stonefly *Tyrrhenoleuctra* [34,41]; younger lineages

accumulate substitutions at a relatively faster pace (yet slower than that of other insect orders). In the only case where nuclear DNA sequences were used (land snails *Solatopupa*, histone *H3* gene; [39]), these were not evolving in a clock-like manner.

Taxa ¹	Linearity	Gene partition	Rates
Land snails <i>Solatopupa</i>	Yes	12S, COI 1 st codon pos.*	0.131, 0.025
Aquatic cave Isopods <i>Stenasells</i>	Yes	15 allozymic loci	2-2.1
Aquatic cave Isopods <i>Stenasellus</i>	Yes	COI all pos., COI Tv+Ti 3 rd codon pos.; COI Tv3 rd codon pos.	1.25, 0.1, 0.46
Terrestrial Isopods <i>Helleria</i>	Yes	COI all codon pos.**	N/A
Stoneflies <i>Tyrrhenoleuctra</i>	Yes	11 allozymic loci	0.8
Stoneflies <i>Tyrrhenoleuctra</i>	No	12S, COI all codon pos.	0.01-0.25, 0.09-0.79
Cave beetles <i>Ovobathysciola</i> <i>Patriziella</i> <i>Anillochlamys</i>	Yes	COI all codon pos., COI 3 rd codon pos., COI Tv3 rd codon pos.	1.3, 0.86, 0.5
Cave beetles <i>Speonomus</i>	Yes	COI all codon pos., COI 3 rd codon pos., COI Tv3 rd codon pos.	1.2, 0.98, 0.9
European newts <i>Euproctus</i>	Yes	12S + 16S all sub., 12S + 16S Tv, Cytb all codon pos., Cytb Tv all codon pos., Cytb Tv3 rd codon pos.	0.22, 0.04, 0.38, 0.08, 0.22
Bediagra rock lizard <i>Archaeolacerta</i>	No	ND4, tRNA ^{SER, LEU, HIS}	2.74, 1.78

¹ Earthworms are not shown here because [35] did not test the molecular clock hypothesis.

*16S, COI 2nd and 3rd codon positions and *H3* did not pass the molecular clock test. ** 12S and 16S not tested for linearity of rates.

Table 3. Summary of molecular rates for Corsica- Sardinia- Iberian Peninsula lineages. The first column shows whether substitution rates passed a molecular clock test; the second and third columns give the data as partitioned in the original study and the relative rates of substitutions. Rates are given as percentages of substitutions per site per lineage per million years for all partitions but for allozymes where rates are in percentages of genetic divergence *D* [66] per lineage per million years.

Even though *COI* rates are relatively similar in our dataset, we are by no means implying that these rates could be carelessly applied to other organisms and/or geographic contexts. There are at least two aspects that we think deserve attention; the time-slice we are looking at and the geographic setting. Deep nodes of a phylogeny often suffer from saturation of sequences; choosing the appropriate model of sequence evolution is then crucial to incorporate saturation in the estimates [42]. If sequences are not behaving in a clock-like manner, methods should be used to accommodate acceleration and deceleration of rates along the branches of a given phylogenetic tree without the need to clear the data set of the non-clock data [43,44]. As we move closer in time, the problem of the discrepancy between times of gene and population divergence arises. This is because prior to species divergence, a degree of gene divergence has already accrued in the ancestral species gene pool. This ancestral species divergence can be a large fraction of the total species divergence if the ancestral species was highly structured and, depending on the size of the ancestral population, could impact the first several million of years after divergence [42]. A way to get around this problem is to adopt a genealogical coalescent-based approach in the data analysis because this can robustly take into account the stochastic genealogical component to divergence [45]. Insularity is usually seen as a simplification when it comes to estimate divergence times. Species divergence, however, might precede isolation due to insularity. In other words, species age might be older than island age. If so and the island age is still used as calibration point, we would end up with biased estimates of molecular rates. Galàpagos and Barbados are examples of insular settings harbouring lineages older than the extant islands [46,47].

Figure 3 shows the molecular age estimates for the peri-Tyrrhenian groups considered in the present study arranged by the major geological events that affected the area.

It should be noted that those groups with a significant TreeMap analysis (aquatic subterranean isopods, stoneflies and newts) have ages consistently closer to the older geological estimates of splits of landmasses than groups with a non-significant TreeMap analysis have. The implication is that gene flow within these groups was discontinued at the geological onset of geographical barriers (detachment between the microplate from the Iberian Peninsula and split between the two islands, respectively). For those groups whose distribution includes the Tuscan Archipelago and/or coastal areas of Central Italy, divergence is relatively young in agreement with the recent interaction between those areas and Corsica. The only exception to this otherwise generalized pattern is *Hormogaster* (earthworms). For this group, allozymes indicate the split between Sardinian and Iberian lineages as coeval with the split between Sardinia and Tuscan Archipelago/Central Italy (17.5-13 Myr). If this hypothesis were true, this would imply that gene flow among these lineages ceased sometimes in the Middle Miocene. At the time, Corsica and Sardinia were either completely detached from the continent (classic scenario Figure 1) or connected through a land bridge to the emerging Italian peninsula (alternative scenario; inlets in Figure 1). Since we have to exclude both oversea dispersal for evident ecological reasons and human-mediated transport (genetic divergent would be much lower in that case) we should give credit to the land bridge hypothesis. Alternatively, and perhaps more parsimoniously, we think that the lack of any Corsican population in the data set is responsible [35]. The island is likely to host lineages phylogenetically intermediate between Sardinia and the Tuscan Archipelago/ Central Italy [48].

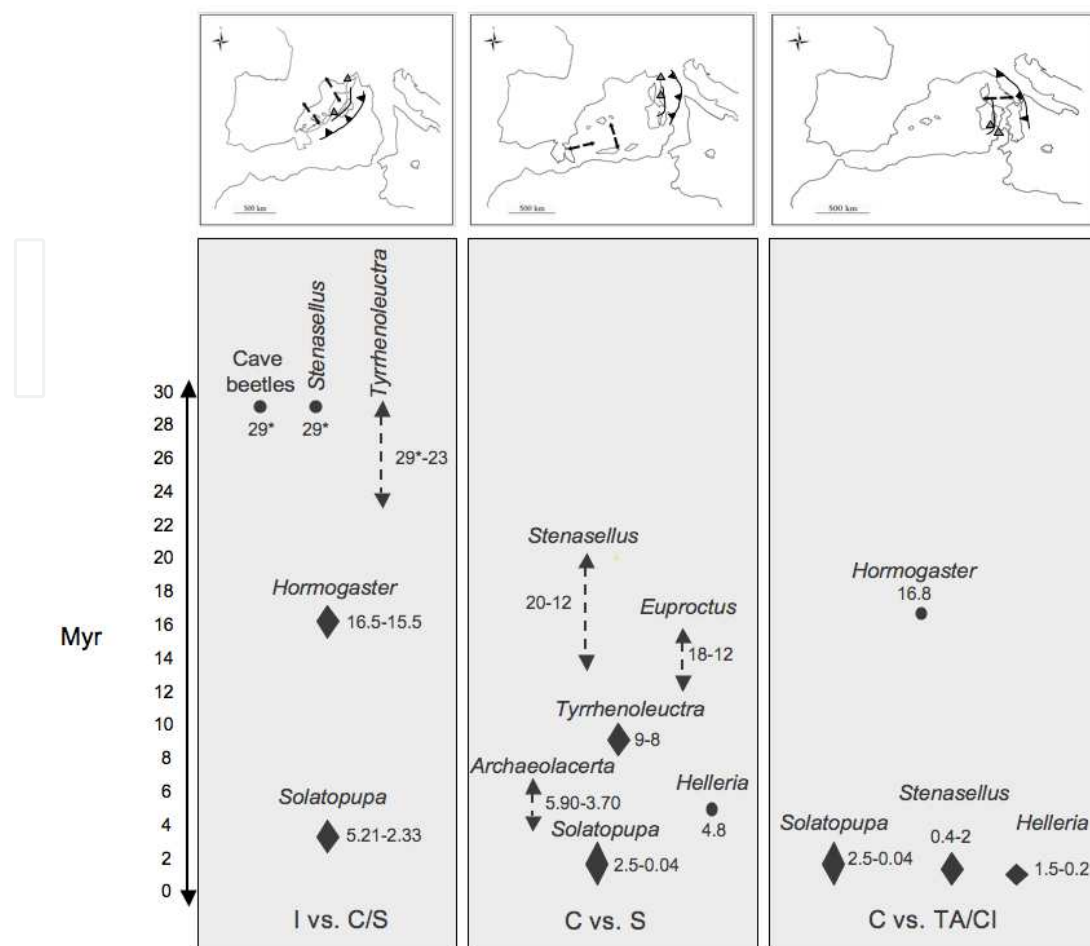


Figure 3. Within taxonomical group molecular age estimates of cladogenetic splits. Splits are sorted according to the three major geological events in the area (I vs. C/S: detachment of the microplate; C vs. S: separation of the two islands; C vs. TA/CI interaction between Corsica and coastal Tuscany). Age ranges (when available in the original source) are shown; asterisks indicate that a particular dating was used to calibrate substitution rates. The three uppermost panels illustrate the geological settings of the three events and are simplified versions of panels (b), (d), and (e) of Figure 1.

Comparisons between Corsican and Tuscan (both insular and continental) populations of *Helleria brevicornis* gave very young age estimates and a pattern of relationships without a visible geographic component [26]. MtDNA genealogy, coalescence inferences and distribution pattern (the species occurs spottily in Tuscany regardless of the abundance of suitable habitats) suggest historic, human-mediated transport as responsible, possibly due to the intense commercial trades existing in the area when the Tyrrhenian Sea was under Etruscan control [26].

8. Comparisons to other insular systems

All the molecular studies reviewed here consistently support a monophyletic origin for the Corsican-Sardinian lineages, regardless of what the relationships within the system are. Table

4 summarizes the main findings deduced from the evolutionary literature available for both continental and oceanic islands.

Island system	Origin	Age (Myr)	Minimum distance from the continent	Connection with the continent	Colonization		Radiation/ Adaptive radiation
					Single (S)/ Multiple (M) Process	Vicariance (V)/ Dispersal (D)	
Canary Islands ¹	Volcanic	21-1	110 km (Africa)	No	S/M-D		Yes/?
Hawaiian Islands ²	Volcanic	29-0.40	3000 km (North America)	No	S/M-D		Yes/Yes
Galàpagos Islands ³	Volcanic	4-0.5	960 km (South America)	No	S/M-D		Yes/Yes
Chatham Islands ⁴	Continental	70	800 km (New Zealand)	No	S-V		Yes/?
Madagascar ⁵	Continental	88	400 km (Africa)	Yes	S/M-V/D		Yes/Yes
West Indies ⁶	Mixed*	48-20	81 km (North America)	Partial	S/M-D/V(?)		Yes/Yes
Philippine Islands ^{7,8}	Mixed*	28-2.5	800 km (Asian mainland)	Partial	M-D		Yes/Yes

* West Indies are of continental origin but Lesser Antilles are mostly volcanic. Philippines are the result of tectonic and volcanic activity and progressive uplift.

References: ¹[52]; ²[53]; ³[47]; ⁴[51]; ⁵[28]; ⁶[55]; ⁷[54]; ⁸[67].

Table 4. Summary of phylogeographic reviews available on other insular systems. For each of them information on the origin, age (extant islands only), minimum distance from and past connection to the continent are given. The two last columns indicate whether insular lineages originated through single or multiple events, via vicariance or dispersal and whether there is evidence of within-system radiation and/or adaptive radiation.

We are aware that a crude comparison between islands of different origin would be meaningless, because intimately different are the ancestry and evolution of the respective biota. Remote oceanic islands can only be colonised through dispersal while both vicariance and dispersal play a role in determining the biological diversity of continental islands [49,50]. The contribution of either factor is related to a large extent to the dispersal ability of organisms. Vicariance is expected to be predominant in poor dispersers (as also shown for the Corsica-Sardinia system) while such a force would be less relevant (if not negligible) in those lineages with a strong vagility. Comparing the studies listed in Table 4 requires additional caution because the molecular data sets they are based upon are quite unbalanced in terms of taxonomic coverage. Hawaii, Galàpagos, Madagascar and West Indies have been covered from a

fair to a deep extent. This is not the case for the Chathams where only a few taxa have been considered.

Keeping in mind the above considerations, these studies show that a vicariant, monophyletic origin can be assumed only in the case of the four genera of large flightless insects from the old continental Chatham Islands (cockroaches, crickets and beetles) analyzed by [51]. About 60% of the invertebrates and 20% of the vertebrates Madagascar harbours have an ancient (i.e. Gondwanian; about 80 Myrs old) vicariant origin. On all the oceanic systems, lineages derived from multiple colonization events co-exist with lineages originated through single founding episodes. Multiple lineages of Canarian reptiles were established via independent episodes of colonization, while darkling beetles, brimstone butterflies and fruit flies reached the archipelago only once [52]. Some representatives of the extant terrestrial fauna of the remote archipelagos of Hawaii and Galàpagos, perhaps the best studied oceanic insular settings, derives from single colonizing episodes while for others molecular data do not justify such an assumption [47,53]. Groups with a monophyletic origin include some evolutionary paradigms such as the Hawaiian drosophilids and honeycreepers and the Galàpagos giant tortoises and Darwin's finches. The past physical connection, although partial, with the mainland of both West Indies and Philippine Islands facilitated colonization [54,55]. Intriguingly, West Indies have been identified also as source of colonization for the surrounding continents and not only as a sink [55].

Frequently lineages diversify on islands. Local lineage production can be repeated many times resulting in a radiation; radiation is sometimes associated with adaptation (adaptive radiation; Table 4). Table 1 shows that Corsica and Sardinia generally host lineages that have diversified locally. It shouldn't be overlooked, however, that only a few studies are based on a dense sampling of populations. We hence suspect the true number of genetic lineages to be underestimated. Keeping in mind the limitations in terms of sampled populations of the studies listed in Table 1, it should be noted that the highest number of detected lineages within either Corsica or Sardinia is seven (cave beetles; [25]). This figure is well below the estimates reported in Table 4, which in some cases exceed 50 (i.e. Hawaiian honeycreepers). Diversification on Corsica and Sardinia was certainly triggered when they became detached from the continent. Subsequent within-island evolution has been documented molecularly but very often it did not produce appreciable morphological differences. Illustrative is the case of the subterranean isopod *Stenasellus* [33,40,56]. The few known populations of this crustacean are virtually indistinguishable morphologically and yet they are deeply divergent from one another at both mitochondrial and nuclear loci.

Given the available data it is impossible to argue in favour of a Corsica-Sardinia radiation, let alone adaptive radiation. Corsica and Sardinia are not as isolated as the majority of the other insular systems listed in Table 4. Also, they are considerably younger than Madagascar and the Chathams, continental islands that witnessed radiation (with adaptation for Madagascar). Finally, it is not unrealistic to think that when the two islands started moving away from the continent they were not as species-poor as typically are young oceanic islands, which provide plenty of ecological opportunities for immigrants. The number of fossil and extant taxa that can be brought back to the initial phases of insularity of Corsica and Sardinia (see the Ecology

and Endemism section) do not depict these islands as blank slates available for colonization and subsequent diversification but rather like hosting already structured biota.

9. Conclusions

Studies conducted so far on organisms with a peri-Tyrrhenian distribution have confirmed the area as one of primary interest for evolutionary research. It offers, in fact, the opportunity to test hypotheses in a well-defined biogeographical context due to the uniqueness of its fauna and the detailed knowledge of its past geological evolution. Available molecular data, along with the analyses carried out *de novo* for this review, suggest that diversification was predominantly driven by vicariance. Allopatry can be safely assumed for organisms strictly bound to freshwaters (both superficial and subterranean; crustacean isopods, stoneflies and newts). Nonetheless, we do believe that these researches have only started scraping the surface of a scenario that is emerging as more complex than previously thought.

The studies reviewed here were meant to unveil relationships at the species or even at the genus level; therefore sampling designs were rarely conceived to disentangle processes below those levels. The few studies designed at the population level failed in retrieving one-landmass one-monophyletic-lineage associations (land snail *Solatopupa guidoni*, terrestrial isopod *Helleria brevicornis*, Bediagra rock lizard *Archaeolacerta bediagrae*) [26,27,37]. This suggests that dispersal could dim the historical signal even in taxa that are apparently not well equipped for substantial movements over long distances. The number and kind of molecular markers used in relation to the evolutionary timescale they are trying to target is yet another critical issue. Most of the studies reviewed here are based on a single locus (often mtDNA). Such an approach works well to resolve old splits, but as one moves towards more recent events the information content of a single locus (which provides us with a gene tree) is rapidly blurred by the random noise typically associated with stochastic population processes. Hence, the discordance between what we have in hands (a gene tree) and what we should aim to (the species tree) is maximized.

From what emerges from this and other reviews (Table 4), it is evident that an accurate understanding of evolutionary processes on islands could be better attained when co-distributed taxonomically independent taxa are investigated in a comparative manner. For the Corsica-Sardinia system we are already in a good position because the work done so far in the area has already identified a number of species that could be used for the scope and for which we have fairly accurate phylogenetic reconstructions. We would now need samplings at the population level to maximize the likelihood to retrieve an accurate representation of their evolutionary histories. Studies conducted on insular endemisms (i.e. Galápagos tortoises, *Anolis* lizard) [47,55] have taught us that size of the island and within-island potential barriers to gene flow correlates positively with number of evolutionary independent lineages. In the case of Sardinia, the phylogeographic structure of the endemic carabid beetle *Percus strictus* [57] was found to accurately reflect the subdivision of the island into three separated landmasses at the beginning of the Pliocene. This potential source of genetic regionalism shouldn't be overlooked when planning further genetic studies. Ideally, samplings should include multiple populations to contrast genetic structuring on either side of the putative barriers to that across them.

Accurate sampling at the population level should then be coupled with a thorough screen of multiple molecular markers to minimize the gap between gene and species trees. This is being made easier by the escalating availability at reduced costs of high-throughput second-generation sequencing. Only until a few years ago, avoiding the limitations idiosyncratic to the single locus approach (as that applied to most of the peri-Tyrrhenian organisms reviewed here) would have required considerable investments in terms of both working time and financial resources. Nowadays, we are in the position to easily isolate batteries of highly polymorphic nuclear markers (microsatellites) [58]. Multi-locus Single Nucleotide Polymorphisms (SNPs) are emerging as even more powerful tools than microsatellites to infer structure of natural populations [59] and they are becoming increasingly popular as the technical challenges associated with their optimization subside. Thousands of SNPs can be identified in a relatively easy manner by using high-throughput sequencing of restriction-site-associated DNA tags (RAD tags); these markers have proved able to supply resolution sufficient to infer patterns of population relatedness [60]. For Corsica-Sardinia organisms we could take advantage of the molecular phylogenies already available. These could be used as guidelines to develop SNPs that are fixed or nearly fixed within populations but variable among them [61]. The genome-wide sample of genotype data is likely to overwhelm most of the sampling error (if any) and, hence, to produce better estimates of phylogeographic relationships without any prior investment in genomic resources being necessary.

The flourishing of new methods to harness large numbers of tailored-to-the-scope molecular markers has proceeded in parallel with (and partially stimulated) the development of sophisticated phylogeographic analytical tools [62]. At the same time, the rise of the coalescence theory is causing a shift in the treatment of phylogeographic data from exploratory to model-driven [62,63]. In an exploratory framework, phylogeographic inferences are based on qualitative interpretations of (often) single-locus gene genealogies. Molecular data, coupled with external information such as species ecology and landscape context, are used directly to infer the demographic history of taxa. This approach is necessary when an *a priori* phylogeographic hypothesis for the taxon (or the area) of interest is not available and has to be generated anew. In the fortunate circumstance that such *a priori* hypotheses exist, alternative scenarios could be discriminated statistically and the one that fits the data best be chosen [64]. In doing that we obviously restrict ourselves to a subset of the whole possible scenarios but with the advantage to accurately estimate key components of the species demography and history of divergence ([62] and references therein). Under a model-driven approach, gene genealogies are not anymore central to the phylogeographic analysis but they rather represent variables for connecting data to demographic parameters under an explicit statistical coalescent model [63]. The peri-Tyrrhenian area offers a unique opportunity to use the model-driven approach and to test its strengths and weaknesses because its geological history is well understood and relatively simple, thus restricting considerably the number of possible alternative phylogeographic scenarios, granted that the appropriate taxon is chosen (i.e. poor disperser). A further advantage is that the insular condition is per definition simpler than any continental one and, hence, complexity of models could be reduced at limited costs in terms of model misspecification risk.

About twenty years of molecular work on these fascinating Mediterranean islands have unveiled their potential as yet another natural laboratories for the study of evolutionary processes. This review, besides summing up what has already been done, wants to stimulate further research in the area. With both the methodological and analytical progresses that evolutionary biology has witnessed in recent years, it is not difficult to envision the Corsica-Sardinia system as an exceptional playground to investigate phylogeographic patterns at an unprecedented level.

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