Predicted sea-level changes and evolutionary estimates for age of isolation in Central Mediterranean insular lizards

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Abstract

Rates of biological evolution on islands are often presumed to exceed rates on the mainland. We tested this postulation by computing the evolutionary rate of head shape in Italian wall lizard *Podarcis siculus*, occurring on four islands off the coast of Southern Italy. We calculated the evolutionary rate using a phylogenetic tree whose node ages were derived from Lambeck et al. predicted ages of geographic isolation of the islands. Such ages are based on a relative sea-level change model for the late Pleistocene–Holocene. Through a likelihood optimization procedure, our method allows computing, besides the evolutionary rate, biological estimates of the ages of insular populations, with this indirectly testing Lambeck et al.'s model estimates. We found that the rate of evolution in *Podarcis* head shapes on islands is not statistically different from the mainland rate, although insular lizards have distinctive head shapes. Overall, the insular phenotype took 1–4000 years to arise (differing among islands). The estimated ages of insular populations are lower than Lambeck et al.'s estimates and fall in the 5- to 6-ka interval.

Introduction

Islands are renown to harbor unusual life forms, from Darwin's finches to dwarf elephants (Whittaker and Fernández-Palacios, 2007), so that the evolutionary process there has always attracted considerable interest by the scientific community. The changes in insular organisms are related to almost any aspect of the phenotype, from behavior to shape, and species ecology. As compared with mainland relatives, species on islands are known to develop different life history strategies (Adler and Levins, 1994; Blondel, 2000; Raia et al., 2010b), extensive body size change (Foster, 1964; Lomolino, 2005; Raia and Meiri, 2006), physiological adaptations to the peculiar insular environment (Herrel et al., 2008; Pafilis et al., 2011), and low gear locomotion (McNab, 1994; Ricklefs and Bermingham, 2002). The main determinants of such changes are thought to be size, diversity, isolation, and age of the insular environment, which, in turn, dictate the ecological conditions the insular population will evolve into (Whittaker and Fernández-Palacios, 2007).

While insular evolution is given so much attention, the physiographic island characteristics, and the time evolution has had to operate on the organisms there, are less often investigated. Time to isolation is especially important because phenotypic changes are influenced by species diversity on the island (Foster, 1964), and it takes some time for phenotypic change to take place. Still, to calculate the rate of evolution, time has to be known, but, in fact, it is usually inferred from phylogeny. This could be severely misleading because unrecorded past extinctions alter branch lengths causing wrong rate estimation (Raia and Meiri, 2011; Thomas et al., 2009), and the time to colonization could be different from the age of island formation.

Local geomorphological studies and regional to global geophysical models now allow time to island final emergence to be estimated with confidence from predictions of relative sea-level (RSL) changes (Lambeck et al., 2011), at least when other processes (e.g. tectonics, erosion) are not operating. For this study, the considered period for the modern insular separation broadly spans the last ~18 ka (late Pleistocene–Holocene), when the recent sea-level rise occurred following the Last Glacial Maximum (LGM) eustatic low at ~120 m below the present sea level (Lambeck et al., 2011, and references therein). RSL is influenced by three main factors, measured with respect to the mainland: (1) the

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sea-level response to the past glacial cycle, including the response to the glacial unloading of the major ice sheets and the response to the ocean floor loading by the melt water (the glacio-hydro-isostatic contribution); (2) global changes in ocean volume from thermal expansion, recent glacier melting, and so on (the eustatic contribution); and (3) the regional- to local-scale vertical land movements (the tectonic contribution). The isostatic response requires the use of models for the past ice sheets, which are estimated from inversions of rebound data from the formerly glaciated regions, and rheological parameters that may be regionally variable, estimated from the sea-level data for sites that are known to be tectonically stable.

Lambeck et al. (2011) provided predicted sea-level curves for the Central Mediterranean Sea since the LGM at 40 reference sites, both tectonically stable and unstable, that are representative of the Italian coast. The model curves take into account the specific isostatic response of the crust to removal of glacial masses both in the northern hemisphere and in the Alps and to the load of the released water column on the Italian continental shelves.

For the Italian coast, tectonic stability is assumed where the Last Interglacial (LIg) shoreline (which traces back to ~125 ka) occurs 7 ± 2 m above present sea level. Where the LIg shoreline either occurs above or below this level, it means that the area has been subjected to uplift or subsidence, respectively (Ferranti et al., 2006). Thus, the solutions are based on an iterative process in which the rheological parameters are tested against the tectonically stable sites and these parameters are then used across the region to estimate the departures from stability.

The model predictions have a wide range of application, including estimation of vertical land displacement at tectonically unstable sites – the regional to local contribution to RSL changes and projection for future sea-level rise at strategic sites (Lambeck et al., 2011; Lambeck's model hereafter). Although the model has been benchmarked against a wealth of geomorphological, stratigraphic, and archeological data, constraints are at places sparse and a validation based on an independent line of observations would be desirable. On the other way around, the predicted sea-level curves may themselves offer a possibility to test hypotheses regarding biological evolution on islands. Much pertinent here, since Lambeck's model offers a reliable estimate of the time elapsed since the formation of an island (in terms of its separation from the adjacent coastline), its predictions can provide a reference to test the long-held idea that evolution is faster on islands (Chiba, 1999; Clegg et al., 2002; Herrel et al., 2008). Positive evidence in favor of this notion was commonly found in studies analyzing the changes in body size in insular species (Lister, 1989; Millien, 2006). Since insular faunas are often species-poor and several ecological niches therefore empty, insular colonizers may evolve at unbounded rates toward either large or small sizes (Foster, 1964; Lomolino, 2005). Yet, insular species do not track any body size (Raia et al., 2010a), the direction of size change is not always predictable (Meiri et al., 2008; Raia and Meiri, 2006), and the size of insular inhabitants is less extreme than expected by chance, when few exceptional cases are considered in the context of the entire insular diversity (Meiri et al., 2011). Even the idea that the rate of evolution on islands is faster has been empirically falsified in studies about Caribbean *Anolis* lizards (Thomas et al., 2009) and mammals (Raia and Meiri, 2011). Actually, accelerated rates of evolution on islands may just be a statistical artifact depending on how rates are calculated (Gould, 1984; Pérez-Claros and Aledo, 2007; Sheets and Mitchell, 2001). Yet, the question remains open, as extreme rates are presumed to be common in small islands at least (Millien, 2011; Thomas et al., 2009).

Herein, we test whether evolutionary rates are faster on islands and seek to compare Lambeck's model predictions to biologically derived time estimates. To these aims, we investigated the morphological evolution in head shape in the lizard *Podarcis siculus*

Figure 1. Location of study sites. The two *Podarcis siculus* body color patterns are shown.

from four small islands on the Tyrrhenian Sea margin of Campania region, Southern Italy (Figure 1). Our study sites include both very small and moderately large islands with either rough or gentle topography. Moreover, we selected sites that are tectonically stable. The advantage of studying tectonically stable sites resides in the possibility of discarding recent land motion, which is an episodic and spatially variable process and may seriously and unpredictably affect the model test.

Still, rather than focusing on body size alone, we analyzed multivariate phenotypic changes in melanic *P. siculus* insular populations (Raia et al., 2010b). Using shape rather than univariate dimensions is desirable because the changes in the phenotype are inherently complex; hence, taking a simple measurement to represent the phenotype could be misleading (Simpson, 1953).

Materials and methods

Study species

The Italian wall lizard (*P. siculus*, Rafinesque 1810) is present in much of the Mediterranean basin as result of its highly invasive profile (Crnobrnja Isailovic et al., 2009). Several insular *P. siculus* subspecies differ from mainland individuals in color and body size, most conspicuously by presenting a blue (melanic) coloration. In Campania Region, where the islands considered here are located, insular subspecies (*P. siculus coeruleus, P. s. klemmeri, P. s. monaconensis, P. s. salfii*) show such melanic coloration (Corti et al., 2011). The blue color was eventually demonstrated to depend on the so-called reversed island syndrome (RIS; Raia et al., 2010b), which is a suite of character shifts including increased aggressiveness and intense sexual dimorphism (Raia et al., 2010b) that run opposite to the changes predicted by the Island Syndrome. Blue lizards are quite common on islets (such as the Faraglioni cliffs off Capri island), while on larger islands (such as Capri itself) lizards present the usual coloration with green trunk and pale undersides.

Geomorphological aspects of the study sites

We selected one island (Capri) hosting lizards with normal body coloration, and four islets settled by blue lizards, at documented stable coastal sites of the central Tyrrhenian Sea (Figure 1). The tectonic stability of all these sites is proved by the near-eustatic elevation of the LIg shoreline marker (see above), which is represented by a tidal notch carved in the calcareous rocky coast (Ferranti et al., 2006).
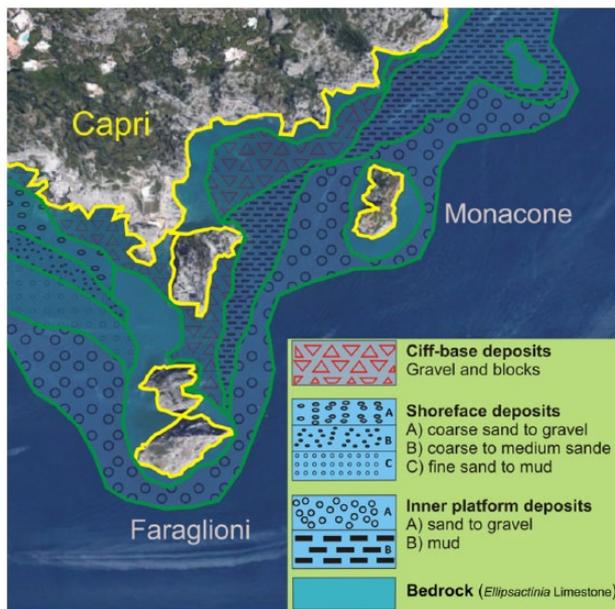
Figure 2. Seabed map showing the depositional textures of the seafloor around Capri, Faraglioni cliffs, and Monacone islet. Source: ISPRA http://www.isprambiente.gov.it/it.

Figure 3. Bathymetric map of the seabed surrounding Capri Faraglioni cliffs.

Two islets (Scopolo, 40°32′24.31″N, 14°15′11.74″E; Monacone 40°32′36.22″N, 14°15′24.24″E) are in the southern part of Capri island ~30 km south of Naples. A third islet (Vetara, 40°34′59.66″N, 14°23′51.14″E) is found nearby in front of the Sorrento Peninsula, and the fourth islet (Licosa, 40°15′04.23″N, 14°54′01.64″E) is located off the Cilento Peninsula ~80 km southeast of Naples (Figure 1). All these islets are relatively close to mainland and are separated from it by a relatively short seaway, with depth not exceeding 80 m. The depth of the water column assures that these islets were connected to mainland during the LGM, and the stretches of land connections were progressively flooded during the following sea-level rise.

In order to estimate the timing of isolation using the model prediction, as discussed in a following section, we measured the distance between islets and mainland and, more importantly, the present minimum depth and the width of the intervening submarine saddle. For two of the islets, Vetara and Monacone, which have the deepest saddle, we extracted this depth from the existing bathymetric and nautical charts. We produced our own bathymetric map for the Faraglioni, using a 33-kHz analogic echo-sounder onboard a light vessel equipped with DGPS positioning (instrumental error <1 m). For Licosa, which has a narrow saddle with a limited operational depth, we measured this depth by means of detailed scuba-diving survey (instrumental error <0.1 m, performed by FC and LF). For these two latter cases, the need of higher accuracy arises because the lesser depth reflects a younger post-LGM flooding, and thus, errors propagate more largely on the isolation age estimate.

Capri island is tectonically stable as suggested by the elevation of the LIg notch, which only documents a very weak tilt toward the north (Antonioli et al., 2015; Ferranti and Antonioli, 2007). The island is separated from mainland by the ~5-km-long Bocca Piccola seaway, which attains a minimum depth of ~−80 m. On the SE side of Capri, three sharp pinnacles or sea-stacks known as Faraglioni are found near the coast (Figure 2). The more landward Faraglione di Terra is connected to land through a wave-cut platform and is home to the *P. siculus* individuals with normal coloration. The Faraglione di Mezzo follows seaward and is separated from the Faraglione di Terra by an ~80-m-long seaway that is shaped as a ~60-m-wide saddle, with a maximum 18 m depth in its ~30-m-long central part (Figure 3). The magnitude of sediment deposition provides a source for uncertainty in age estimation. We therefore performed scuba-diving checks of the lithological nature of the sea-bottom reported in official maps. Such direct observations and existing seafloor maps (Figure 2) show that the saddle is incised in bedrock and is draped by a very thin sheet of gravel, which makes our estimates reliable. The Faraglione di Mezzo is incised midway by an arch-passage with near-vertical sides and a depth of −15 m. The farthest islet, Faraglione di Fuori locally known as Scopolo, is only 10 m away from Faraglione di Mezzo, and the narrow passage has a maximum depth of −8 m in the central part (Figure 3). In summary, the main insular barrier is the ~80-m-long seaway between the Faraglioni di Terra on one side and the two sea-bound Faraglioni on the other side.
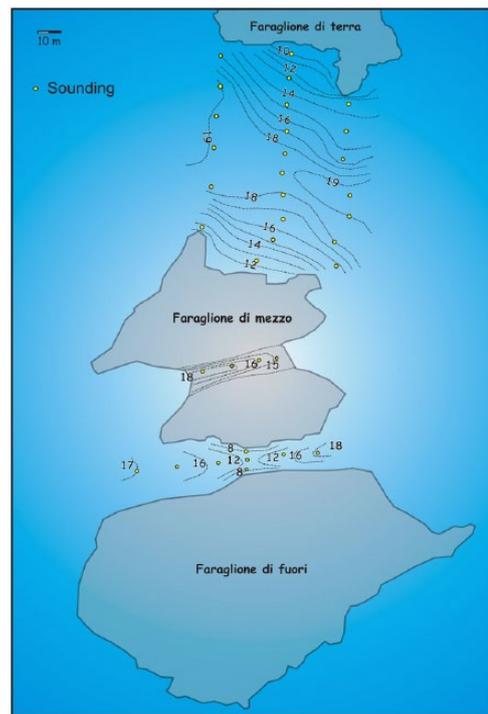
The nearby Monacone (Figure 2) is a more leveled islet compared with the Faraglioni. It is located ~120 m off the coast of Capri, and the intervening, ~100-m-wide saddle is 22 m deep. The saddle is broad and carved in bedrock, with only minor detrital and sandy cover (Figure 2). Both the Monacone and Faraglioni saddles probably result from erosion during one or more still-stands of the sea-level, which, based on global sea-level curves (Waelbroeck et al., 2002), presumably coincide with Marine Isotope Substages (MIS) 5c and 5a dated at ~100 and ~80 ka, respectively.

The Sorrento Peninsula east of Capri is an ENE-WSW horst made of carbonate rocks (Figure 1). Few islets punctuate the southern side of the Peninsula and represent the culmination of tectonically downthrown bedrock blocks. Fault displacement is today ceased as documented by the eustatic position of the LIg marker on the peninsula cliffs (Cinque and Romano, 1990). The Vetara islet is one of these block culminations, located today ~1.5 km off the coast (Figure 4). The saddle between mainland and the islet, as derived from nautical charts, has a maximum depth of ~−45 m. A sandy mud veneer covers the shelf landward of the saddle, which is mostly sculpted in the carbonate bedrock (Figure 4). Submerged terraces remnants carved in the carbonate bedrock, too, are found at −18/−20 m and are correlated to the saddles of Faraglioni and Monacone, further testifying the tectonic stability of the area.
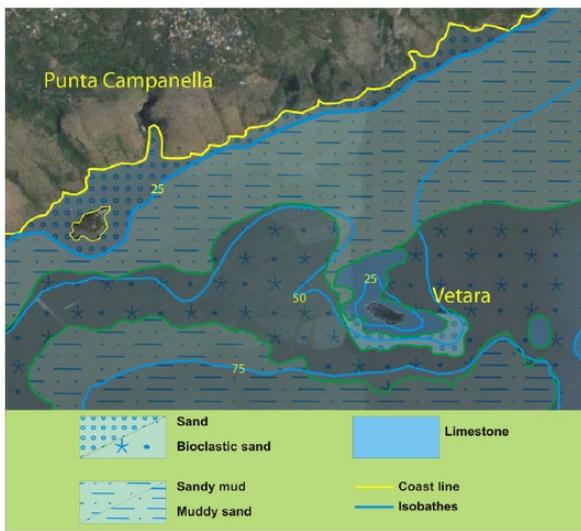
Figure 4. Seabed map showing the depositional textures of the seafloor around Vetara islet.
Source: ISPRA http://www.isprambiente.gov.it/it.

Licosa islet is located at the SW tip of the Cilento promontory (Figure 1). The islet and the adjacent coast are floored by lithified Miocene sandstone and siltstone. A marine terrace carved in bedrock at ~7–9 m elevation along this coast is attributed to the LIg (Iannace et al., 2001), thus accounting for a minimum uplift since 124 ka. The islet lies ~350 m off the coastline and is separated from it by a saddle which rises at a minimum ~5 m depth.

Geometric morphometrics of head shape in P. siculus

We focused on the head shape of *P. siculus* in order to evaluate the rate of phenotypic evolution in insular versus mainland settings and to provide a test for Lambeck's model-predicted sea-level curves.

To this aim, we implemented a database of *P. siculus* head pictures used in Raia et al. (2010b) with additional individuals collected from seven different populations present in the Campania region, during September–October 2015. We sampled two male individuals of the subspecies *P. s. salfii* from Vetara islet and 10 (6 males (M) and 4 females (F)) from mainland relative population of Punta Campanella (40°34′34.06″N, 14°19′45.25″E). We also collected 10 individuals from the population of the Capri's Faraglioni: 3 males from the cliff Monacone belonging at the subspecies *P. s. monaconensis* and 7 (3 M and 4 F) from the Scopolo cliff which pertain to the subspecies *P. siculus coeruleus*. Furthermore, we collected 14 (8 M and 6 F) lizards in Capri island (40°33′3.62″N, 14°14′34.95″E). In total, 45 individuals (16 M and 29 F) were collected on Licosa islet. They belong to *P. s. klemmeri*. Finally, we collected 15 individuals from the coastline facing Licosa (Punta Licosa, 40°15′06.15″N, 14°54′19.68″E). They include 12 M and 3 F of the mainland, normally colored *P. siculus* individuals.

All individuals were caught by noose or Sherman's trap, then transported to the nearby field station using professional climbing techniques to carry out all measurements, and subsequently released. Only adult individuals were included in the study. We considered as adults specimens larger than 45 mm in snout-vent length (SVL), in keeping with Carretero et al. (2006), Henle (1988), and our own personal observations.

After sexing the individuals, we took orthogonal pictures in dorsal view using a Canon EOS 7d DSLR camera with lens Canon EF 100mm f/2.8 L macro IS USM. Each specimen was positioned parallel to the photographic plane in the center of the optical field in order to reduce and equalize distortion. A grid was used as a background for scaling. The camera setup and the placement of each specimen relative to the camera lens were kept constant so as to minimize image aberration because of parallax (image distortion resulting from placing the camera too close to the specimen) and to reduce error in the subsequent analysis (Mullin and Taylor, 2002).

All field work was performed in accordance with the Ethical Committee for Animal experiments, University of Naples 'Federico II' (ID: 2013/0096988), and according to the Italian law (DL 26/2014).

We quantified head shape using geometric morphometrics (Rohlf and Marcus, 1993). This method analyzes anatomical shapes as recorded by a set of coordinates (landmarks) located on the pictures. We used generalized procrustes analysis (GPA) (Rohlf and Slice, 1990) to remove nonshape information because of position, orientation, and scale of the landmark configurations. For each image in dorsal view, we identified 16 landmarks on the left side only of the head to avoid redundant information from symmetric structures (Klingenberg, 2010). All landmarks were digitalized with TpsDig2 version 2.17 software (Rohlf, 2013). The choice of landmarks was based on their presence in all specimens and their reliability in providing an adequate summary of specific aspects of head morphology (see Supplementary Material, available online). The landmark configuration we applied was used in previous studies of head shape variation in lacertid lizards (Urošević et al., 2012, 2013). Landmarks represent either contact points between cranial plates or the point of maximum curvature of the head, in keeping with the homology principle (Kaliontzopoulou et al., 2007).

From the rotated, aligned, and scaled landmark configurations, we extract the aligned coordinates (landmarks' coordinates obtained after GPA) using the TpsRelw version 1.53 software (Rohlf, 2013). This set of coordinates effectively represents head shape variation in our sample and were therefore passed to the analysis of evolutionary rates.

Rates of insular evolution and test of Lambeck et al.'s model estimates

The Brownian motion (BM; Felsenstein, 1973, 2008) is a model of phenotypic evolution describing how phenotypic traits (e.g. body mass, body length) change on a phylogenetic tree (that is a representation of species genealogy). It develops on the idea that phenotypic differences among species accumulate over time at constant rate and variance, while the mean trait value (the phylogenetic mean) remains constant. BM is thus a time-dependent, non-directional process. The rate of this process (σ^2) is proportional to the variance of trait values and inversely proportional to tree height (that is the duration of time evolution has operated). BM is one of a class of evolutionary models used to describe phenotypic evolution. To test for the appropriateness of BM in the context of head shape evolution in insular lizards, we decomposed the head shape data by principal component analysis and then extracted the first axis of shape variation (RW1 in the geometric morphometrics literature). This axis captures the largest share of shape variation in the data. We then fitted the RW1 values against alternative (to BM) models of phenotypic evolution, including models that presume an 'early-burst' mode of shape change followed by stasis.

While evolutionary models such as BM usually compute a single σ^2 for a given trait and phylogeny, there are methods to estimate whether there are differences in the rate of evolution over time and across the tree (e.g. Eastman et al., 2011). Adams (2014) and Denton and Adams (2015) devised a method to quantify phylogenetic evolutionary rates for high-dimensional
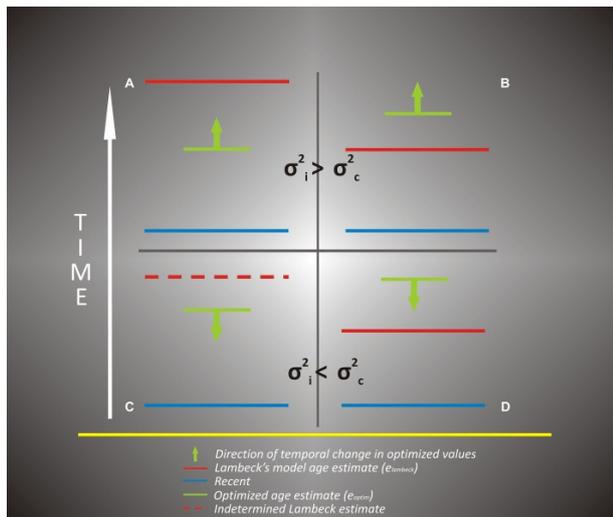
Figure 5. The procedure applied to estimate the age of biological separation of insular populations from the mainland relatives. The four quadrants represent all of the hypothetical combinations of rate of evolution and estimated ages as compared with Lambeck's model age estimates. See text for further explanation.

data, based on distance matrices. It allows calculating distinct multivariate evolutionary rates for different parts of the tree, under the specific assumption that certain clades (groups of species sharing a common ancestor) evolved at different rates. The presumed clade-wise rate differences are then tested against the null that traits evolved according to a single multivariate evolutionary rate (fitted according to BM) across the whole tree.

We used a phylogenetic tree of *P. siculus*, where the ages of nodes subtending to insular individuals are estimated according to a likelihood optimization function. The function minimizes the difference in the rate of evolution of head shapes in insular versus continental individuals. It tells what the age of the 'insular' nodes should be in order to make the evolution of insular individuals head shapes as fast as in continental ones. If head shape evolves faster on islands, the nodes will be pulled back in time to result in a slower actual rate (σ^2) and the other way around if the insular rate of evolution is slower. This provides an effective yet partial test for Lambeck's model-derived ages of separation between islands and the adjacent mainlands, whose interpretation depends on the outcomes of the optimization procedure (Figure 5). The ages of insular nodes are allowed to take any value within a large range of time, encompassing the ages estimated according to Lambeck's model. If the real insular rate σ_i^2 (as calculated with Adams' 2014 method) is higher than the real continental rate σ_c^2 (Figure 5, upper row), the optimization will pull the estimates back in time to level the rate difference out. Otherwise, for $\sigma_i^2 < \sigma_c^2$, the optimization will push the estimates toward the recent (Figure 5, bottom row).

The underlying idea is to compute the real insular and continental rates first, then minimize the difference through optimization, and finally compare the resulting estimates with RSL model predictions. For instance, if head shape evolution is faster on islands and the estimated optimized age (e_{optim}) turns out to be younger than Lambeck's separation age estimate ($e_{lambeck}$), it could indicate that $e_{lambeck}$ could be overestimated. This is because although e_{optim} is allowed, in principle, to get older than $e_{lambeck}$, it remains younger, despite the fact that optimization compensates for the higher σ_i^2. It is not redundant to note that in this fictionary example where $\sigma_i^2 > \sigma_c^2$, e_{optim} is to be taken as the oldest possible value of the estimate, since still larger (i.e. older) e_{optim} would imply $\sigma_i^2 < \sigma_c^2$, which makes no sense.
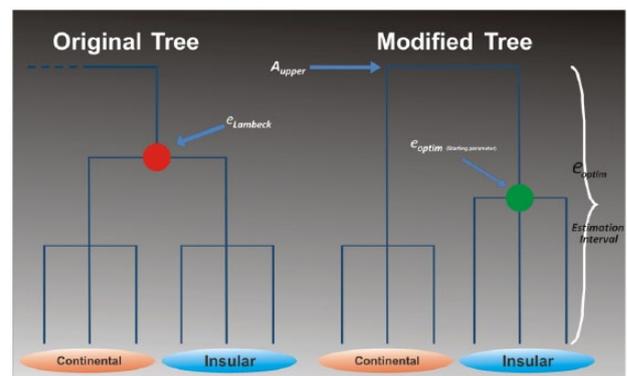
Figure 6. Depiction of the methodology applied to prepare the phylogeographic trees of *Podarcis siculus* populations included in this study. The actual trees are reported in Figures S2, available online (original version) and S3 (modified version).

In particular,

For $e_{optim} < e_{lambeck}$ and $\sigma_i^2 > \sigma_c^2$, $e_{lambeck}$ is overestimated (Figure 5A);

For $e_{optim} > e_{lambeck}$ and $\sigma_i^2 > \sigma_c^2$, $e_{lambeck}$ is underestimated (Figure 5B);

For $e_{optim} < e_{lambeck}$ and $\sigma_i^2 < \sigma_c^2$, there is no meaningful insight about $e_{lambeck}$ (Figure 5C);

For $e_{optim} > e_{lambeck}$ and $\sigma_i^2 < \sigma_c^2$, $e_{lambeck}$ is underestimated (Figure 5D).

Although we use the terms overestimate and underestimate above, we urge the reader to consider that these predictions have to be taken at face value, and by no means they necessarily indicate the age of island formation. We will return on the possible reasons for eventual discrepancies between different age estimates in the 'Discussion' section.

To use the optimization procedure, we had to prepare and then modify an apt phylogenetic tree (Figure 6). The tree was derived from geographic information, so that for each island we took lizards' continental relatives (hence, sister clade) from the closest mainland locale (see details in Supplementary Information, available online). The age of insular clades initially corresponds to Lambeck's estimates ($e_{lambeck}$ Figure 6, red dots). To estimate e_{optim}, we modified the tree as follows. Let A_{upper} be the age of the node parental to $e_{lambeck}$, so $A_{upper} > e_{lambeck}$. In the modified tree, the separation between insular and mainland sister clades corresponds to A_{upper}. For the sake of the optimization, the age of the most recent common ancestors of the insular individuals is set initially at $A_{upper}/2$ (Figure 6, green dots), but is estimated in the interval between 0 (the recent) and A_{upper}.

Results

RSL-predicted age of insular isolation

Here, we used the dataset from Lambeck et al. (2011) and additional material to generate two predicted sea-level curves for the study sites. The two curves were built after several sites in Campania (sites 9 and 26 in Figure 1 of Lambeck et al., 2011: Map of Italian coast with location of the sites with predicted sea-level curves in Appendix 1), which are representative of Licosa and of Scopolo, Monacone, and Vetara islands, respectively. As shown in Figure 7, the two curves are similar to a slightly higher RSL for Licosa as compared with Capri because of minor differences in glacio-hydro-isostatic adjustment between the two sites. Both
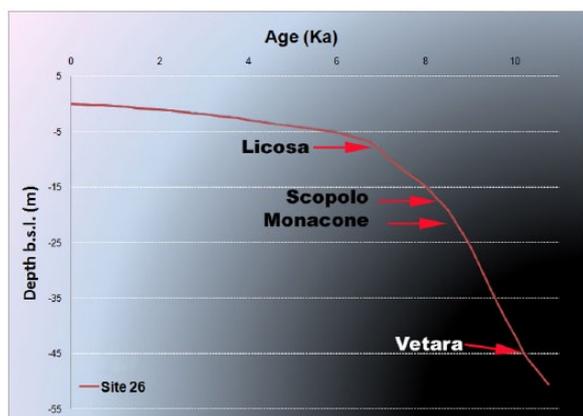
Figure 7. RSL curves computed for Licosa, Monacone, Scopolo, and Vetara islets. Arrows point to predicted age of geographic isolation for each islet.

curves predict a rapid sea-level rise from 9 to 6.8 ka and a progressive decrease in the rate of rise from 6.8 ka onward.

Because of the documented lack of Holocene tectonic displacements, as illustrated in section 'Study species', the predicted geographic isolation for the study sites is directly drawn from the two curves at the depth of the saddle separating each island from mainland (Figure 7).

Clearly, an unspecified error in the estimated isolation age derives from the model parameters and from the spatial projection of the study sites relative to sites 9 and 26. We regard these errors as adding little uncertainty because, particularly for the latter process, of the similarity of the two curves.

Besides errors embedded in the choice of model parameters, seafloor erosion by marine currents and sediment deposition contribute to the uncertainty in the estimation of the age of islets isolation. These counteracting processes, though, are of very minor importance in our study settings. In fact, our direct observations confirm that at Capri, Vetera, and Licosa, the seafloor at the saddle is mostly carved in hard carbonate bedrock, with only a minor gravelly cover (Figures 2 and 4), and thus, post-flooding sediment aggradation does not add significant uncertainty. As concerns erosion, we have no direct control on uncertainty magnitude. However, given the relatively narrow sea passages, strong marine currents induced by the Venturi effect are unlikely. In addition, significant mechanical erosion during the rapid late-Pleistocene–early-Holocene sea-level rise is commonly thought to be negligible. Mechanical erosion rates in clastic rocks flooring the Messina Straits between Calabria and Sicily during the last sea-level rise are estimated at 0.1 mm/yr (Antonioli et al., 2014), and in the less-erodible carbonates flooring three of the islets (Faraglioni, Monacone, and Vetara), and also in the hard sandstone at Licosa, they are certainly lower. Thus, we conclude that this source might imply a <1-m error at most in the depth estimation of the paleo-saddle.

Figures 8 and 9 portray a simplified reconstruction for Capri and Vetara, respectively, showing the paleogeographic modifications, ultimately leading to the current isolation that occurred at specific time frames during the last sea-level rise. We recall here that the Faraglioni existed as islets well before the Holocene and at least before 125 ka, when the LIg notch was sculpted all around them. The products of progressive destruction of the spur from which the Faraglioni are the last standing portion form a narrow band of cliff-base gravel that marks the former extent of the spur (Figure 2). During the post-LGM sea-level rise, the Faraglioni area of Capri starts to be singled out as a headland between ~10 and 8.5 ka (Figure 9a and b), whereas nearby Monacone is already a separate islet (Figure 9c). It
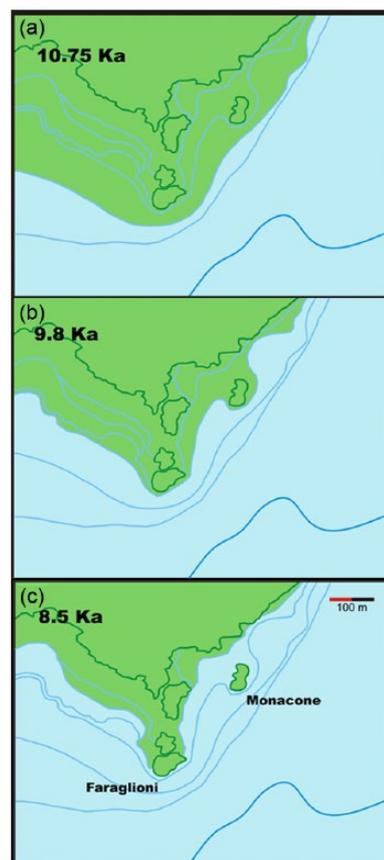
Figure 8. Paleogeographic reconstructions for the Faraglioni area during the 10.75–8.5 ka time interval, showing progressive stages leading to island formation: (a) 10.75 ka, (b) 9.8 ka, and (c) 8.5 ka.

probably took only few thousand years to recreate, at ~8.2 ka, the present Faraglioni isolation.

Vetara is individuated as a spur between ~13.5 and 11 ka (Figure 9a and b), before being cut off from mainland at 10.2 ka (Figure 7). From then onward, the islet experiences a progressive albeit limited reduction in areal size (Figure 9c).

Evolutionary rates and comparison between evolutionary and RSL age estimates

We found a small difference between the real rate values, with $\sigma_i^2 > \sigma_c^2$. Their ratio is 1.06 and not significant ($p = 0.306$; Table 1).

The likelihood optimization function effectively made the ratio equal to 1. In all four cases, the estimated ages of nodes subtending to insular individuals (e_{optim}) are younger than Lambeck's model estimates ($e_{lambeck}$). The difference is as much as 4 ka (Table 1).

Discussion

The main results of this study are that (1) the insular shape evolution is by no means faster than the continental one, contrary to the conventional wisdom; (2) the estimated age of isolation of insular lizard populations on the study islands tends to collapse within a relatively small (~1 ka) interval; and (3) these ages are systematically younger than ages predicted by the RSL rise model.

As for the first result, our data comply with earlier reports finding no significant difference in rates of evolution on islands (Raia and Meiri, 2011; Thomas et al., 2009). Millien (2011) specifically predicted that accelerated rates pertain to small islands. In our study sample, we included islets as small as cliffs off Capri island (e.g. Scopolo, area = 14,000 m²; Monacone,

area = 11,000 m^2) and the islets of Licosa (area = 9000 m^2) and Vetara (area = 28,000 m^2). Yet, no statistically significant difference emerges. This is particularly relevant since these islets harbor populations of melanic lizards whose phenotype strongly differ from their mainland relatives. This phenotypic shift has been epitomized under the RIS and includes increased aggressiveness, increased famine, and intensity of sexual selection and, more relevant here, increased sexual dimorphism in head shape in insular melanic individuals (Novosolov et al., 2013; Raia et al., 2010b). This result is consistent and does not depend on the choice of any particular evolutionary modes, because decomposing head variation by means of principal component analysis indicates BM is actually the best description of head shape variation in our study lizards (Table 2).
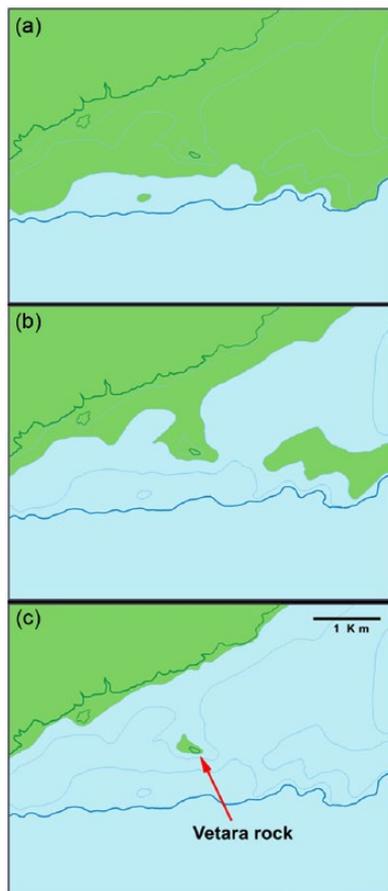
Figure 9. Paleogeographic reconstructions for Vetara area during the 13.5–9 ka time interval, showing progressive stages leading to island formation: (a) 13.5 ka, (b) 10.7 ka, and (c) 9 ka.

As regards the second result, the estimated ages of isolation are similar to each other and cluster in the 5–6 ka BP interval, notwithstanding significant differences in the RSL rise-predicted isolation ages at the various study sites (Figure 7).

The third result of this work is that the four e_{optim} estimates we calculated are younger than the corresponding $e_{lambeck}$ values and differ from each other by as much as 4.2 ka. In fact, we found that σ_i^2 is slightly larger than σ_c^2, which would indicate that $e_{lambeck}$ is overestimated. Yet, this difference is not statistically significant. Furthermore, as we cautioned above, it is important noticing that $e_{lambeck}$ is an estimate for the physical separation of an island from the continent, while we argue e_{optim} is best understood as referring to age of genetic isolation of the insular population from the mainland. It is reasonable to assume that for some time after the physical separation of an island, continental individuals continued to land on the island. Lizards could be good dispersers, especially because their low metabolism allows sustaining long periods of famine abroad rafting branches, which provided the most obvious mean of colonization of the islets we dealt with here. The influx of continental individuals in the insular population might have halted or considerably slowed phenotypic change on the island by disrupting selection toward any derived insular condition. This implies that e_{optim} should be younger than $e_{lambeck}$ even if $e_{lambeck}$ is correct. It is impossible to know island-by-island how long mainland individuals may have continued to contribute to the genetic pool of the insular population. Yet, the difference between e_{optim} and $e_{lambeck}$ is always large (Table 1b), suggesting that either $e_{lambeck}$ estimates are in fact too large or that the colonization of the islands occurred quite some time after the islands formed. An intriguing, third alternative is that although the rate of evolution is not larger on islands than on the mainland, island phenotypes could have remained stable after the insurgence of RIS. One observation coming from the results for the Capri cliffs (Scopolo and Monacone) supports this idea. Scopolo and Monacone are very close to each other (Figure 2), but have different $e_{lambeck}$ estimates. Yet, the difference in age between e_{optim} and $e_{lambeck}$ (3.2 ka) is the same for the two islands. Given the close proximity between the cliffs, colonization rate from Capri Island should coincide for the two, which would suggest the 3.2-ka difference between e_{optim} and $e_{lambeck}$ just indicates the (maximum estimate for) time to completion of RIS after colonization. Although such conclusion is fascinating and opens up the way for systematic comparison at other sites, we call for more robust confirmation of it.

It could be argued that if a delay exists in the reality, the comparison between evolutionary and RSL predictions does not represent a test for the Lambeck's model. We note that what is important here is not that the equality of the evolutionary and RSL estimate of age separation, but the result that evolutionary ages are always younger than predicted ages. Observational constraints for the Lambeck et al. (2011) curves, even in tectonically

Table 1. Multivariate evolutionary rates for islands (σ_i^2) and mainlands (σ_c^2) *Podarcis siculus* head shape.

	Real	Optimized	
σ_i^2	1.7794E−05	1.6636E−05	
σ_c^2	1.6642E−05	1.6640E−05	
σ ratio	1.0692	1.0002	
	Licosa	Scopolo	Monacone	Vetara
$e_{lambeck}$	6.000	8.200	8.700	10.200
e_{optim}	4.980	4.950	5.560	5.990
Δ_e	1.020	3.250	3.140	4.210

Real: real rates; optimized: rates optimized as to keep $\sigma_i^2 = \sigma_c^2$; $e_{lambeck}$: ages estimated with Lambeck's model (in kiloannum); e_{optim}: ages estimated through optimization under the condition $\sigma_i^2 = \sigma_c^2$; $\Delta_e = e_{lambeck} - e_{optim}$.

Table 2. Likelihood and Akaike information criteria for models of evolution of *Podarcis siculus* head shape.

	Log-likelihood	AIC	AICc	Free parameters
BM	189.05	−374.10	−373.97	2
Lambda	193.98	−381.95	−381.69	3
Delta	192.42	−378.85	−378.58	3
EB	189.05	−372.10	−371.84	3
Trend	191.20	−376.41	−376.15	3
OU	193.80	−381.59	−381.33	3

AIC: Akaike information criterion; AICc: penalized AIC; free parameters: number of fitted parameters; BM: Brownian motion.

quasi-stable sites such as those studied here, come for the Holocene time span mostly from sediments drilled in coastal plains and secondarily from geomorphological and geoarcheological markers scattered along the rocky coasts of Italy. Along the very rugged cliffs of the locales studied here, poor or no Holocene constraints are available, and thus, the independent upper bound (the genetic isolation) for geographical separation provided in the study represents a confirmation of model reliability.

Conclusion

In this study, we analyzed a number of blue-colored, insular lizard populations showing emergence of the so-called RIS (Raia et al., 2010b). We found that shape evolution in insular individuals is not faster than on the continents, although previous studies have suggested this to be the case.

The estimated age of isolation of the blue lizards on the study islets is comfortingly lower than geomorphological estimates. More interestingly, these biological age estimates cluster within a small temporal interval, suggesting that despite the diversity of island systems, age, and independent colonization events, the insurgence of the RIS takes comparable time to take place. The intersection of purely geological and evolutionary approaches and methods proved a successful tool to study biological evolution in islands.

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