



# Incorporating evolutionary processes into conservation planning using species distribution data: a case study with the western Mediterranean herpetofauna

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## ABSTRACT

**Aim** To incorporate evolutionary processes into conservation planning using species distribution patterns and environmental gradients as surrogates for genetic diversity.

**Location** Western Mediterranean Basin.

**Methods** Distributions of 154 herpetological species were predicted using maximum entropy models, and groups of significantly co-occurring species (biotic elements) were identified. Environmental gradients were characterized for the complete area and for the area covered by each biotic element, by performing a principal component analysis on the data matrix composed of nine environmental variables. The first two principal component analysis axes were classified into four categories each, and those categories were combined with each other resulting in an environmental classification with 16 categories. To identify priority conservation areas, biotic elements and environmental categories were used as surrogates for the neutral and adaptive components of genetic diversity, respectively. Priority areas for conservation were identified under three scenarios: (1) setting targets for species only; (2) setting targets for species and for each environmental category of the overall area; and (3) setting targets for each species and for each environmental category within each biotic element.

**Results** Nine biotic elements were identified – four for the amphibians and five for the reptiles. Priority areas identified in the three scenarios were similar in terms of amount of area selected, but exhibited low spatial agreement.

**Main conclusions** Prioritization exercises that integrate surrogates for evolutionary processes can deliver spatial priorities that are fairly different to those where only species representation is considered. While new methods are emerging to incorporate molecular data in conservation prioritization, it is unlikely to be enough data for enough taxa for this to be feasible in many regions. We develop an approach using surrogates for both the neutral and adaptive components of genetic diversity that may enhance biodiversity persistence and representation when molecular data are not available or geographically comprehensive.

## Keywords

Adaptive variation, biotic elements, environmental gradients, genetic diversity, neutral variation, spatial prioritization.

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## INTRODUCTION

In the current global biodiversity crisis (Pimm & Raven, 2000), prioritizing conservation areas that maximize species repre-

sentation and enable persistence by maintaining key ecological and evolutionary processes is a major goal of conservation biology (e.g. Brooks *et al.*, 1992; Margules & Pressey, 2000; Desmet *et al.*, 2002; Moritz, 2002; Rouget *et al.*, 2003).

However, conservation planning has tended to focus more on biodiversity pattern (representation) than on the evolutionary processes that generate and maintain biodiversity (Pressey *et al.*, 2007). To maximize the probability of species persistence, conservation practitioners should incorporate knowledge on evolutionary processes and the distribution of genetic diversity in conservation planning (Crandall *et al.*, 2000; Moritz, 2002; Neel, 2008).

Several methodologies have been suggested to incorporate evolutionary history and processes in conservation planning. For example, phylogenetic diversity (PD) (Vane-Wright *et al.*, 1991; Faith, 1992) is a biodiversity index that measures the length of evolutionary pathways that connect a given set of taxa. By using appropriate algorithms (e.g. Rodrigues & Gaston, 2002; Steel, 2005), it is possible to choose a subset of taxa that maximizes representation of evolutionary distinctiveness. In general, in a balanced phylogenetic tree, PD would be higher if one selects a set of taxa from branches separated by the main splits in the tree, i.e. by selecting sample taxa of the main phylogenetic lineages (Avisé, 1992; Rodrigues & Gaston, 2002). These main lineages are probably the result of extensive historical isolation periods, such as vicariance events, derived from allopatric speciation (Avisé, 1992). While this approach would maximize the representation objective of conservation planning, by including maximum genetic diversity, it has been criticized on the grounds that it disregards areas of recent diversification, where adaptive genetic variation is highest, driving phenotypes or ecological traits, and therefore influencing retention of individual fitness and population viability (Smith *et al.*, 2000, 2005). Consequently, it has been recognized that both the neutral and the adaptive components of genetic diversity need to be preserved to achieve conservation of evolutionary processes (Brooks *et al.*, 1992; Moritz, 2002).

One of the challenges of integrating evolutionary processes in conservation planning derives from conservation planning being a spatially explicit process, which makes it necessary to spatially identify the neutral and adaptive components of genetic diversity. Previous studies have made progress to circumvent such challenge. For example, some authors (e.g. Moritz & Faith, 1998; Smith *et al.*, 2000) used comparative phylogeography to identify sets of species sharing a common vicariance history, and subsequently used Venn diagrams to identify areas representing multiple range restricted unique lineages. Another approach identified areas with higher concentrations of lineage breaks to spatially reveal endemism at the subspecific level (Rissler *et al.*, 2006). In parallel, the adaptive component of genetic diversity has been addressed by identifying areas where recently diverged endemic species co-occur, as a proxy for rapid diversification areas or evolutionary hotspots (Davis *et al.*, 2007; Vandergast *et al.*, 2008) and by modelling patterns of genetic and morphological variation (Thomassen *et al.*, 2010, 2011). One important consideration about these studies is that they focused on a limited number of taxa, to which molecular data were available. However, molecular data are still lacking or is very incomplete for most taxa, and available information is often collected using

different molecular markers and statistical methods, which hinders integration into consensual phylogenies for multiple taxa. Therefore, using spatial surrogates for evolutionary processes is likely to be essential in conservation planning in the near future.

One of the possible surrogates for the neutral component of genetic diversity is community structure. Comparative phylogeography studies show that species with similar ranges often tend to be genealogically structured in similar ways (Avisé, 2000, 2009). The explanation for this finding is that the emergence of geographic barriers can lead to vicariance and subsequent allopatric speciation of several taxa (Hickerson *et al.*, 2010). Consequently, deeply separated phylogroups are often confined to biogeographical regions as identified from current species assemblages (Avisé, 2000; Crisci *et al.*, 2003). In other words, the vicariance model predicts that the ranges of the species originating by the same vicariance events will be, on average, more similar to each other (Hausdorf, 2002). A classic example where genetic surveys have provided strong evidence for phylogeographic concordance is represented by the Mediterranean Peninsulas which constituted Pleistocene refugia for many taxa (Taberlet *et al.*, 1998; Hewitt, 2000; Weiss & Ferrand, 2006). Thus, in areas where vicariance events played an important role in structuring biotas, the identification of groups of species with significantly co-occurring ranges may be a surrogate for the neutral component of genetic diversity.

Regarding the adaptive component of genetic diversity, some studies have used ecological and climatic gradients as surrogates (e.g. Cowling *et al.*, 2003; Rouget *et al.*, 2003). The rationale for using these surrogates is that ecology plays a major role in speciation and adaptive radiation in many natural populations (Smith *et al.*, 2005) because environmental gradients facilitate genetic differentiation and character displacement (Doebeli & Dieckmann, 2003). Thus, adaptive speciation is expected to be greatest along steep environmental transitions (Smith *et al.*, 2001; Budd & Pandolfi, 2010; Thomassen *et al.*, 2010), which often coincide with areas of high beta diversity, where different species assemblages co-occur (Spector, 2002; Graham *et al.*, 2009). However, these areas with high beta diversity often constitute marginal areas of species' ranges, where overall genetic diversity within species populations is generally lower (see Emerson & Gillespie, 2008 for a comprehensive review). Thus, the recommended conservation strategy is to protect contiguous habitats of sufficient area across major environmental gradients within the overall species assemblages, including the core and marginal areas of species' ranges (Araújo, 2002; Moritz, 2002; Pleguezuelos *et al.*, 2008; Smith & Grether, 2008).

In this study, we aim to incorporate evolutionary processes into conservation planning strategies using current patterns of species distributions and environmental gradients as surrogates. To do so, we identify groups of significantly co-occurring species – biotic elements (as surrogates for the neutral component of genetic diversity), characterize environmental gradients within such groups (as surrogates for the adaptive component), and use a reserve selection algorithm to

identify priority areas for conservation. We compare these results with those obtained without incorporating surrogates for evolutionary processes.

We focus our study on the herpetofauna of western Mediterranean because it is a 'natural laboratory', where multiple historical events are thought to have triggered cycles of vicariance events followed by dispersion towards climatic gradients. Important historical events included the Messinian salinity crisis (MSC), which occurred around 5.96 Ma (Hsü *et al.*, 1977; Krijgsman *et al.*, 1999; Duggen *et al.*, 2003; Rouchy & Caruso, 2006). The MSC resulted from a complex combination of tectonic and glacio-eustatic processes which progressively reduced the seaways from deep sea to shallow waters (Krijgsman *et al.*, 2000; Braga *et al.*, 2003) and finally isolated the Mediterranean Sea from the Atlantic Ocean (5.9–5.33 Ma) (Krijgsman *et al.*, 1999). This isolation precipitated a relatively fast drying of the Mediterranean Sea, resulting in several land connections between Africa and Europe, allowing dispersal of terrestrial herptiles between the two continents (Pleguezuelos *et al.*, 2008). Around 5.3 Ma, a new series of tectonic activity broke the land bridge between Africa and the Iberia, opening the current Strait of Gibraltar, and allowing the Atlantic ocean to surge into the Mediterranean (Hsü *et al.*, 1977; Rouchy & Caruso, 2006). After the refilling of the Mediterranean, the Strait of Gibraltar became once again a geographical barrier to the dispersal of Iberian and Moroccan biotas.

Other important events in shaping the distribution of current biotas were climatic oscillations. Cold conditions prevailed during most part of the Quaternary Period, but this climatic pattern was periodically interrupted by short periods of rapid climate warming. Molecular evidence suggests that several European taxa contracted their range into the Mediterranean Peninsulas during the ice ages, where the climate was warmer and more humid (Hewitt, 1996; Taberlet *et al.*, 1998; Weiss & Ferrand, 2006). The Iberia, in particular, was one of the most important glacial refugia in Europe for several taxa (Hewitt, 1996). The high topographic and climatic heterogeneity of the Iberia and its habitat diversity favoured the occurrence of multiple glacial refugia isolated from each other, enabling allopatric speciation. These periods were followed by post-glaciation dispersion of population towards northern latitudes and lower altitudes (Hewitt, 1996), tracking climate warming during the interglacials. For amphibians and reptiles, in particular, phylogeographic studies indicated refugia concordance for different species in the Iberian Peninsula, mainly located at the major mountain systems (Gómez & Lunt, 2006).

Given the history of the study area, we expect to find a strong vicariance signature by identifying at least some distinct biotic elements on each side of the Strait of Gibraltar. We also expect to find a spatial congruence between different biotic elements and particular climates within each side of the Strait. The vicariance signature should be quite detectable in amphibians, given their low vagility, while it is expected that dispersion along climatic gradients to be more evident in reptiles, which have higher dispersal capacity.

## METHODS

### Study region

The study region is located at the western part of the Mediterranean Basin (bounded by 13°9' W to 3°20' E and 27°38' to 43°49' N) and includes the continental territories of Portugal, Spain and Morocco (see Appendix S1 in Supporting Information).

### Species and environmental data sources

Species distribution data were collected from the atlases of Portugal (Loureiro *et al.*, 2008), Spain (Pleguezuelos *et al.*, 2002) and Morocco (Bons & Geniez, 1996), other scientific publications (Fahd & Pleguezuelos, 2001; Martinez-Medina, 2001; Carretero *et al.*, 2004; Crochet *et al.*, 2004b; Escoriza & Ortiz, 2004; Fahd *et al.*, 2005; Guzmán *et al.*, 2007) and fieldwork (Harris *et al.*, 2008). The taxonomical list used was the one used in the atlases of Portugal, Spain and Morocco, with a few exceptions: (1) *Triturus marmoratus* and the recently described *Triturus pygmaeus* were treated as one taxon because the distribution data for *T. pygmaeus* were not available for Portugal; (2) we applied the same criteria to species from the genus *Pelodytes* for the same reason. To Iberian species which taxonomical nomenclature changed recently, but there was not a change in the biological unit to which they refer, nor in its corresponding distribution, we updated the nomenclature according to Carretero *et al.* (2009). To all species recently split into two or more species, we maintained the nomenclature used in the atlases, with the exception of *Iberolacerta monticola*, which split into three allopatric species had already been recognized when this project started: *I. monticola*, *Iberolacerta martinezricai* and *Iberolacerta cyreni* (Comisión de Taxonomía de la AHE, 2005). However, the recently described *Iberolacerta galani* was considered together with *I. monticola* given that only recently they were recognized as different species (Carretero *et al.*, 2009). Thus, a total of 153 herpetological taxa were considered in this study (Appendix S2), of which 136 occur on only one side of the Strait of Gibraltar and 18 occur on both sides of the strait. Forty-five species are endemic to the study region. The study area was divided into 12,730 cells using a 10 × 10 km grid. Species distribution data were assembled in a geographical information system.

Sixteen variables known to be related to amphibians and reptiles ecology (e.g. Rodriguez *et al.*, 2005; Soares & Brito, 2007; Whittaker *et al.*, 2007) were compiled from multiple sources. Climatic and topographical variables were downloaded from the WorldClim database (Hijmans *et al.*, 2005), including, temperature annual mean (TMEAN), temperature seasonality (TSEAS), maximum temperature of warmest month (TMAX), minimum temperature of coldest month (TMIN), temperature annual range (TAN), annual precipitation (PAN), precipitation of wettest month (PWET), precipitation of driest month (PDRY), precipitation seasonality

(PSEAS) and altitude (ALT). The variable aspect (ASP) was derived from altitude using ArcGis *Spatial Analyst*. Annual (ANDVI), Spring (PNDVI), Summer (SNDVI), Autumn (ANDVI) and Winter NDVI (WNDVI) variables were downloaded from the Desertification Information System for the Mediterranean (<http://dismed.eionet.europa.eu/>). Pairs of correlated variables were resolved by starting eliminating the variables which were correlated with a highest number of other variables. We followed this criteria until we had no pairs with a Pearson correlation coefficient higher than 0.75. This procedure resulted in excluding for subsequent analysis ASP, TMEAN, and all NDVI related variables, and using in the model calibration the remaining nine variables. The resolution of all variables was converted to a grid cell size of  $10 \times 10$  km to match with the species distribution data, using the average value inside each grid cell.

### Species distribution modelling and patterns of species richness

The sampling effort to collect species occurrence data was probably not homogeneous. For example, for species occurring on both sides of the Strait, the density of occurrence data in the Iberian Peninsula is generally much higher. Consequently, many locations in Morocco have very little species distribution data. Given this, and because the type of input data can influence the identification of significantly co-occurring species (Moline & Linder, 2006), we used a maximum entropy modelling approach with software MAXENT (Phillips *et al.*, 2006) to predict the distribution of each species in less-surveyed areas. This modelling technique requires only presence data as input, but consistently performed well in comparison with other methods (Elith *et al.*, 2006). The average number of occurrence records per species was 943, ranging from 20 to 6347. Taxa with less than 20 occurrence records (30 taxa) were not modelled and only observed records were used in subsequent analysis (Appendix S2). Most of these taxa are local endemics with very restricted ranges.

To build the models, we used the nine variables described earlier. Given that the Iberian Peninsula was much better sampled than Morocco, we reduced the number of occurrences in the Iberian Peninsula for species occurring on both sides of the Strait of Gibraltar to avoid sampling bias. A minimum convex polygon was drawn around each species' occurrence in Morocco to calculate the occurrence density for each species. Then, presence data in the Iberian Peninsula were randomly selected to match the density in Morocco.

To run MAXENT, we used the default 'auto features' option, the recommended values for the convergence threshold ( $10^{-5}$ ) and the maximum number of iterations (500). We set the random test percentage to 20% and the regularization value to 1. We used logistic output, which assigns a probability of occurrence of each species to each cell in the study region (Phillips & Dudik, 2008). We ran the models ten times for each species to avoid bias resulting from randomly splitting the data into training and testing and selecting background points. The

final model for each species was an average of the ten models, a method considered to increase significantly the accuracy of species distribution models (Marmion *et al.*, 2009). We evaluated the accuracy of each model by plotting a receiver-operating characteristic curve (Zweig & Campbell, 1993) and calculating the area under the curve (AUC).

We reclassified MAXENT outputs (relative probability of each species occurrence in each grid cell) to zero (species absence) or one (species occurrence) by determining a species-specific threshold. The threshold was calculated according to the 10 percentile method, i.e. the threshold value corresponds to the model probability where 90% of the occurrence records with the highest model probabilities are presences. To avoid over-prediction, for all species that only occur at one side of the Strait, we converted the probability of occurrence to zero at the side where the species is not known to occur. After predicting the potential distribution of each species, we intersected the individual species maps to derive maps of potential species richness for amphibians, reptiles and total species.

### Biotic elements

We identified significantly co-occurring species groups of amphibians and reptiles in the western Mediterranean region by using the biotic elements methodology proposed by Hausdorf (2002). Biotic elements are defined as groups of species whose ranges are significantly more similar to each other than to those of taxa of other groups. We assume that such groups probably have similar ecological traits and are also likely to share a common biogeographic history (Morrone, 2001; Hausdorf, 2002).

The biotic elements methodology is implemented in the program package PRABCLUS v 2.1.2 (Hennig & Hausdorf, 2008), which is an add-on package for the software R (R Development Core Team 2008). We first tested whether there is a significant non-random congruence of species ranges using function *prabtest*. To do so, we calculated the *kulczynski* distances between each pair of species ranges because it is the most appropriate for data sets characterized by large differences in species ranges (Hennig & Hausdorf, 2004; Moline & Linder, 2006). Subsequently, we calculated the *T* statistics, as the ratio between the 25% smallest and the 25% largest *Kulczynski* distances (Hennig & Hausdorf, 2004). The value of this statistics ranges from 0 to 1, and it is expected to be smaller for spatial clustered data than for homogeneous distributed data. To test whether the clustering is significant, the *T* statistics of the original data should be contrasted with the *T* statistics of species' distributions under a null model, which simulates species distributions in which all inhomogeneities (clustering) of the data can be attributed to the range size distribution, to varying numbers of taxa per geographic unit and to the spatial autocorrelation of the occurrences of a taxon (see Hausdorf & Hennig, 2003 for mathematical details). The significance of the clustering can be verified if the *T* statistics for the original data is smaller than a significant



number of null models. The computational procedure to build the null models is implemented in the function *prabtest* and has been described elsewhere (Hennig & Hausdorf, 2004). The resulting *P*-value is calculated as the number of simulated *T* statistics values higher than the *T* statistics of the original data +1 divided by the number of simulations +1. In our study, we used 500 simulations for the amphibians and 250 for the reptiles.

If there was a significant non-random congruence of species ranges, we determined species biotic elements by using the function *prabclust*. This function uses a distance matrix to produce a multidimensional scaling from the distances. Subsequently, it uses a model-based Gaussian clustering with 'noise' approach to define the clusters; support to determine the meaningful number of clusters is provided by the Bayesian Information Criteria. The *prabclust* function also integrates an initial estimation of noise, allowing identifying species whose ranges cannot be assigned to any biotic element. As a distance matrix, we used the same distance matrix calculated using the *kulczynski* index that was used to test if there is a significant non-random congruence of species ranges. The number of nearest neighbours to determine the initial noise estimation was set to 1 for the amphibians and to 4 for the reptiles, to be proportional to the number of species in each dataset (as suggested by Hennig, personal communication).

### Environmental gradients within biotic elements

To identify environmental gradients within each biotic element and the overall study area, we performed a principal component analysis on the data matrix composed of the nine environmental variables used to build the species distributions models and the grid cells covered by the overall distribution of the species attributed to each biotic element. This way, we decreased the variability of the nine variables to two principal components (PC), explaining a relatively high proportion of total environmental variability. Each component was then classified into four categories, according to quartiles of each axis. Subsequently, each grid cell was classified according to the combination of the categories obtained in PC1 and PC2, resulting in 16 categories for each biotic element. The classification codes were composed of two digits, which coded PC1 and PC2's categories, respectively. For example, code '12' stands for the combination of category one on the first axis and category two on the second axis of the PCA.

### Priority areas for conservation

To identify priority areas for conservation, we used software MARXAN (Ball & Possingham, 2000; Ball *et al.*, 2009). MARXAN uses a simulated annealing algorithm to configure areas that minimize the sum of the cell's costs while ensuring that targets set for each conservation feature are met. We identify priority areas for conservation under three scenarios: setting targets for species only (Scenario Sp); setting targets for species and for each environmental category of the overall area (Scenario

SpEnv); and setting targets for each species and for each environmental category within each biotic element (Scenario SpEnvBE). In each scenario, we aimed to represent 10% of the area covered by each species and/or environmental category while minimizing the total area needed to achieve those targets. Scenario SpEnvBE aims to assure that representation of species distributions within biotic elements is distributed across the environmental variability of each biotic element and thus targets both the neutral and the adaptive components of genetic diversity.

In each scenario, we ran a preliminary sensitivity analysis to determine the minimum species penalty factor (spf) that would assure that all targets were met. We set this value as five for scenarios Sp and SpEnv and as 10 for scenario SpEnvBE. We also conducted a sensitivity analysis to identify a boundary length modifier (BLM) value that would retrieve solutions with a reasonable level of compactness. We set BLM to five in all scenarios after visual inspection of results obtained with BLM ranging from one to 10. Iterations per simulation were set to 10,000,000, temperature decreases per simulation to 10,000 and the initial temperature and cooling factor to adaptive.

Because MARXAN retrieves near optimal solutions, we run it 100 times for each scenario and identified the best out of the 100 solutions as the one achieving lowest score for the MARXAN objective function. We also identified the number of times that each cell was selected out of the 100 runs, hereafter called selection frequency as it is a measure of how frequently a cell is among the 100 good solutions the MARXAN algorithm found.

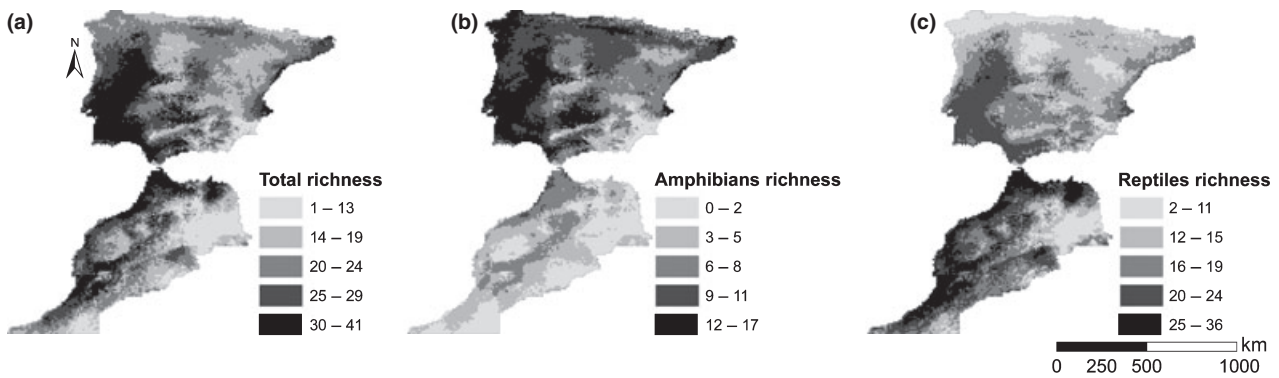
We analysed congruence between solutions by calculating the Pearson correlation coefficient between selection frequencies obtained with each pair of scenarios. We also evaluated agreement between the best solutions found in each scenario. To do so, we classified each grid cell in one of two categories (selected/not selected) and calculated Cohen's kappa coefficient of agreement (*k*) between pairs of scenarios, using package 'psy' in software R (R Development Core Team 2008). If pairs of scenarios are in complete agreement then *k* value equals one. If *k* value is less than 1, it means less than perfect agreement between pairs of scenarios. If *k* is negative, it indicates that the pair of scenarios agrees less than would be expected by chance. We followed the same procedure to evaluate the agreement between the best solution of each scenario and the current Protected Areas network (Appendix S1).

## RESULTS

### Predicted species richness

The AUC values obtained in 123 individual-species models ranged between 0.72 and 1.00 (Appendix S2).

Predicted species richness was unevenly distributed in the study area (Fig. 1). Higher total richness (amphibians plus reptiles) was predicted for south-western and north-western Iberia, the Iberian Central System and the Morena Mountains



**Figure 1** Species richness calculated from the predicted distribution model of each individual species for (a) total amphibians and reptiles, (b) amphibians only, and (c) reptiles only. In each map, species richness is classified in five classes and natural breaks were selected to maximize the difference between the classes. Maps shown in WGS 84 projection.

in the Iberian Peninsula. In Morocco, higher total richness was predicted for the Rif and Atlas Mountains and along the Atlantic coast. The correlation between predicted amphibian and reptile richness was low (Pearson  $R = 0.074$ ,  $P < 0.01$ ), indicating that distribution patterns of richness differs among taxonomic groups: Iberia was richer in amphibians, and Morocco was richer in reptiles (Fig. 1).

### Biotic elements

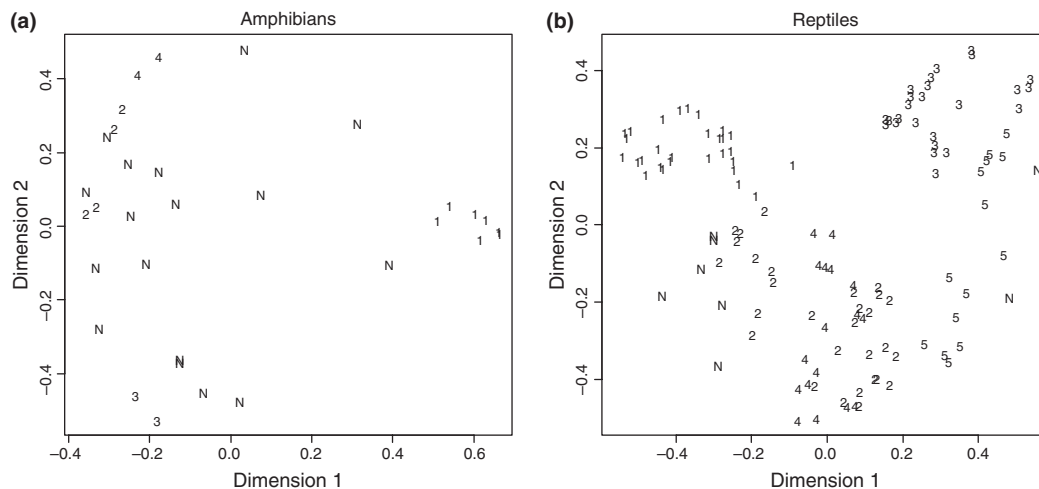
We found that distribution areas of amphibians and reptiles were significantly clustered. The statistic  $T$  was significantly smaller than expected under the null model for amphibians ( $P = 0.001$ , based on 500 simulations) and for reptiles ( $P = 0.008$ , based on 250 simulations).

We determined four biotic elements for amphibians (clusters A1–A4) and five for reptiles (clusters R1–R5), and a noise component for each taxonomic group (Fig. 2). Although clustering was significant, a relatively high proportion of

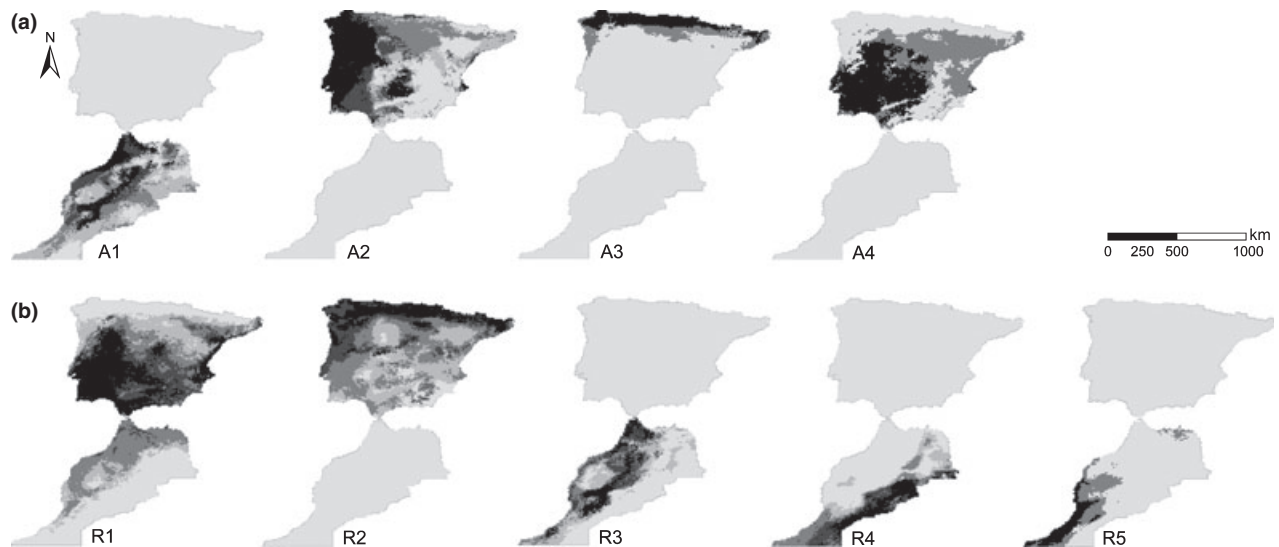
amphibians (53%) and a smaller fraction (7%) of reptiles were not assigned to any biotic element, respectively.

The amphibian biotic elements (Fig. 3a; Appendix S3) included one element comprising species that occur strictly in Morocco (cluster A1) and three elements comprising species that only occur in the Iberian Peninsula (clusters A2–A4). The Iberian biotic elements overlapped to some extent, particularly A2 and A4. However, the core areas of A2 were mainly located in western Iberia while the core areas of A4 were mainly in south-western and central Iberia.

The reptile biotic elements included three groups comprising species distributed at both sides of the Strait of Gibraltar (R1, R2 and R5) and two groups exclusively in Morocco (clusters R3 and R4) (Fig. 3b; Appendix S3). However, it is noteworthy that the two species occurring in Iberia in R5 (*Chamaeleo chamaeleon* and *Testudo graeca*) were introduced by people (Paulo *et al.*, 2002; Fritz *et al.*, 2009). Cluster R1 was largely represented by widespread species, including most of the species with Iberian-Moroccan range. Cluster R2



**Figure 2** Multidimensional scaling of the distribution data of (a) amphibians – four dimensions used, and only first two dimensions shown. Four biotic elements were identified (1–4) plus a noise component (N); and (b) reptiles – three dimensions used, and only first two dimensions shown. Five biotic elements were identified (1–5) plus a noise component (N).



**Figure 3** Geographic distribution of species richness within each of four amphibian (a) and five reptile (b) biotic elements. Species richness is represented in a grey scale, with darker colour indicating higher species richness and white colour indicating absence of species. In each biotic element, species richness is classified in five classes and natural breaks were selected to maximize the difference between the classes. Maps shown in WGS 84 projection.

comprised mostly species distributed along the high-altitude Iberian mountains and also the Rif in Morocco. A considerable amount of spatial overlap occurred between clusters R3 and R5, but cluster R3 had its core area at high altitudes, including the Rif, the Atlas Mountains and the Moroccan Atlantic coastal strip, while cluster R5 included predominantly lower altitude species along Atlantic and Mediterranean coastal areas. Cluster R4 was characterized by species occurring mainly in south-eastern Morocco.

### Environmental gradients within biotic elements

The environmental variability within each biotic element and of the total area was reduced to 16 environmental categories (Appendix S4). The total variance explained by the two axes was higher than 75% in all cases (Appendix S5).

### Priority areas for conservation

The cells identified as priority for conservation differed among scenarios, with respect to both the best solution and the selection frequency map (Fig. 4).

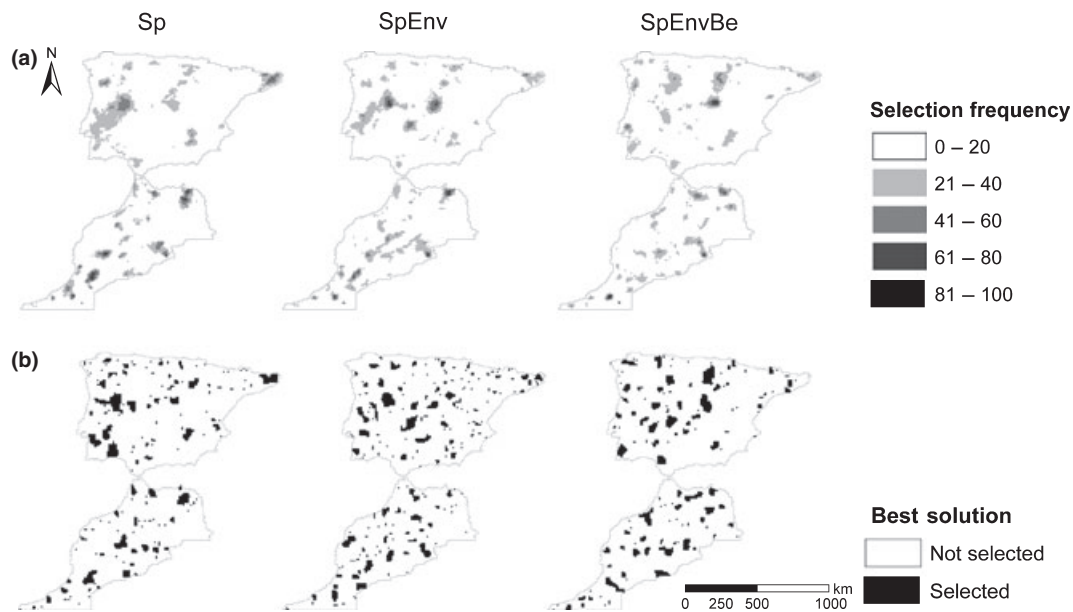
Cells with higher selection frequency identified when targeting only species (scenario Sp) were mainly located along the Tagus river valley, mountains of Peneda-Gerês, Cantabrian, eastern Pyrenees, Guadarrama Mountains, Betic system, and the western section of the Central Mountain System, in the Iberian Peninsula, and along the Moulouya, Ziz, Drâa and Souss river valleys, in particular sections of the Atlases and Rif Mountains and other scattered transition areas in Morocco. Spatial patterns of selection frequency found when targeting species and overall environmental gradients (scenario SpEnv) were relatively similar to those found with Sp, but cells around

the Tagus river valley and the south-western section of the High and the Anti-Atlas Mountains obtain lower selection frequency values, while a section of the Guadiana river valley (east to Merida) and Guadarrama Mountains obtained higher selection frequency values. Spatial patterns of selection frequency obtained when targeting species and environmental variability within biotic elements (scenario SpEnvBE) were considerably different from those found with Sp and SpEnv (highest Pearson correlation coefficient was 0.51, Table 1). Cells with high selection frequency scores were located along coastal northern Portugal, the Esla river valley, northern Iberian system, Rif Mountains and several other smaller regions. Lower selection frequency scores are mainly located along the Tagus river valley, north-east of the Iberia, the Moulouya river valley and the western sections of the Atlases Mountains.

The best solutions found were surprisingly dissimilar among scenarios (Fig. 4) (the maximum Cohen's Kappa coefficient is 0.07, Table 1), although the number of grid cells selected was fairly similar (1134 grid cells in Sp, 1188 in SpEnv and 1288 in SpEnvBE). Agreement between best solutions and the current Protected Areas network (Appendix S1) were also low in all scenarios, being lower than expected by chance in scenario Sp (for scenario Sp,  $k = -0.003$ ; for scenario SpEnv,  $k = 0.063$ ; for scenario SpEnvBe,  $k = 0.007$ ).

### DISCUSSION

This study demonstrates how we can use species distribution and environmental data as surrogates for evolutionary processes in conservation planning. Despite earlier recommendations to explicitly address these processes in conservation strategies (Brooks *et al.*, 1992; Crandall *et al.*, 2000; Moritz,



**Figure 4** Spatial distribution of (a) Selection frequency values and (b) best solution, identified in each MARXAN scenarios: Sp, setting targets for species only; SpEnv, setting targets for species and for each environmental category of the overall area; SpEnvBE, setting targets for each species and for each environmental category identified within each biotic element. Maps shown in WGS 84 projection.

**Table 1** Cohen's kappa coefficients for the pairs of best solutions (upper matrix) and Pearson correlation coefficient for pairs of selection frequency solutions (lower matrix).

	Sp	SpEnv	SpEnvBE
Sp	1	0.07	0.05
SpEnv	0.73	1	0.06
SpEnvBE	0.47	0.51	1

2002), they are generally disregarded (but see e.g. Desmet *et al.*, 2002; Rouget *et al.*, 2003; Klein *et al.*, 2009), probably because of difficulties in identifying spatial surrogates for evolutionary processes.

In the present study, we identified biotic elements and environmental gradients as surrogates for the neutral and the adaptive components of genetic variability, respectively. Our results showed that spatial prioritization exercises that explicitly integrate such surrogates deliver quite different spatial priorities compared to plans that only account for species representation. Moreover, all solution found in each scenario showed limited agreement with the current Protected Areas network. This fact is not surprising given that optimally representing the herpetofauna (or surrogates for its genetic diversity) was probably not one of the objectives of the network design. The most important question here is, how effective are the surrogates used in this study in representing genetic diversity?

The usefulness of biotic elements as surrogates for neutral genetic diversity is limited, because biotic elements can also

originate as a result of post-vicariance dispersal or by other speciation modes (Hausdorf & Hennig, 2004). Thus, caution is needed interpreting our results and it would be useful to integrate them with other biogeographical knowledge. There is evidence in our results that vicariance was not the only process underlying current distribution patterns because (1) the range of different biotic elements overlaps extensively and (2) a large numbers of amphibian species could not be assigned to any biotic element. However, it is noteworthy that species assigned to the noise component of biotic elements were never the non-modelled ones.

Despite these two issues, the biotic elements found in this study are fairly consistent with the most important vicariance events believed to have determined genetic patterns in some of the species. Assumptions based on molecular data (see Appendix S6) corroborate the hypothesis that the current non-random distribution of species is probably derived from both vicariance and dispersal events. Many of the amphibians were classified in the noise component probably because of complex dispersal routes that resulted in species distributions that are quite different, which mask previous vicariance events. The difficulty in finding strong biogeographical patterns for the amphibians in the Iberian Peninsula has been reported before (Busack & Jaksic, 1982; Vargas *et al.*, 1997), although it was attributed to a hypothesized low ecological specialization of the group (Busack & Jaksic, 1982). However, Sillero *et al.* (2009) revealed that both specialist and generalist amphibian species occur in this area, which refutes that hypothesis.

For reptiles, we were able to classify almost all species in significant clusters and to relate them to a few important vicariance events (see Appendix S6). However, we also found



that species within the same genera tended to be classified within the same biotic element, which contradicts the vicariance hypothesis. In fact, species of genera *Iberolacerta*, *Lacerta* and *Podarcis* were predominantly classified in R2, of genera *Blanus* in R3, and *Acanthodactylus*, *Mesalina*, *Spalerosophis*, *Stenodactylus* and *Tarentola* in R4. This fact suggests that either distinct speciation mechanisms other than vicariance are related to species divergence or that post-vicariance dispersal occurred. Nevertheless, molecular studies available to support any of these assumptions are scarce (but see e.g. Fritz *et al.*, 2006).

Cladistic methods based on current species distributions, such as biotic elements or parsimony analysis of endemism, have been criticized for creating misleading interpretations about historical events influencing current species ranges (e.g. Szumik & Goloboff, 2004; Garzón-Orduña *et al.*, 2007). However, in the case of biotic elements, its usefulness goes beyond the identification of groups of species with common biogeographic histories. Determining areas of significantly co-occurring species is relevant from the ecological point of view because it allows recognition of groups of species that share similar niches and respond similarly to ecological disturbances (Marquet *et al.*, 2004; Azeria *et al.*, 2009). Additionally, it allows identification of the core area for the niche of such species, and transitional areas. Such identification is important because environmental characteristics enhancing the probability of persistence, as well as the type and degree of disturbances, differ between biotic elements and thus management and conservation strategies should also differ. For example, species identified under A3 probably have a strong dependence on water, since their range is restricted to areas of high precipitation, while species identified under R4 are probably adapted to xeric conditions. By targeting populations along environmental gradients within biotic elements, we ensure that the overall ecological variability of each niche is preserved, which enhances the probability of persistence under ecological disturbances such as climate change (Pyke & Fischer, 2005). Additionally, targeting ecological gradients among biotic elements forces the MARXAN algorithm to choose grid cells that incorporate both: (1) core areas of species' ranges, where populations' densities are generally higher and more stable and thus more resistant to stochastic threats; and (2) marginal areas of species' ranges, where the genetic differentiation and the adaptation potential are usually higher.

Despite the evidence that environmental gradients facilitate genetic differentiation (Doebeli & Dieckmann, 2003), particularly differentiation of fitness-related traits (Smith *et al.*, 2005), further explicit molecular, morphological and behavioural studies would be required to test such assumptions for all the species targeted in this study and to fully understand spatial patterns of genetic diversity. However, there is evidence that ecological gradients may have influenced speciation. For example, the *Iberolacerta* genus probably diverged from *Podarcis* because of ecological segregation derived from competition, with *Iberolacerta* adapting to high altitudes and

*Podarcis* to the lowlands (Crochet *et al.*, 2004a). There is also evidence that recent lineages of the *Podarcis* species complex may have originated from ecological speciation (Carretero, 2008). Other correlative evidence of genetic and morphological variation with ecological gradients has been reported for many species, mainly justified by post-glacial dispersion towards environmental gradients after climate amelioration (e.g. Alexandrino *et al.*, 2007).

One of the most important challenges in conservation planning for evolutionary process is setting representation targets with ecological meaning. Without explicit molecular data, there is a big challenge to determine how many populations of a species are required to adequately capture within-species diversity (Neel & Cummings, 2003). In this study, we applied the widely used approach of targeting 10% of each taxa distribution. Yet, this method does not specify how many populations (or landscape patches) are necessary for delivering adequate conservation and does not assure that all populations marked for conservation are viable. The question of how much is enough is central in conservation planning but remains largely unresolved (Tear *et al.*, 2005).

We recognize that this study could be improved by incorporating other prioritization criteria, such as threats, connectivity, other ecological processes and socio-economic features. However, our goal was not to identify priority areas to be implemented on the ground, but instead to demonstrate that it is possible to integrate surrogates for evolutionary criteria into conservation planning and that those criteria can deeply influence the selection of the priority sites.

Given the urgent need to make conservation decisions (Grantham *et al.*, 2009), approaches such as the one demonstrated in this study are useful because they enhance the probability of capturing evolutionary processes when molecular data are unavailable or not geographically comprehensive. However, we note that such approaches do not constitute an alternative to studies incorporating molecular data. Recently, there have been several attempts to incorporate evolutionary processes at the subspecific level using molecular data. The neutral component has been addressed, for example, by delimitating evolutionarily significant units (Moritz, 2002), measuring evolutionary distinctiveness (Posadas *et al.*, 2001; Faith *et al.*, 2004; Ajbilou *et al.*, 2006; Forest *et al.*, 2007), or identifying Pleistocene refugia (Carnaval *et al.*, 2009). The adaptive component has been addressed by identifying areas of rapid diversification or evolutionary hotspots (Davis *et al.*, 2007; Vandergast *et al.*, 2008) or modelling relationships between environmental heterogeneity and genetic and phenotypic variation (Thomassen *et al.*, 2010, 2011). However, a comprehensive methodology of how to spatially optimize conservation areas using molecular data for multiple species remains an open challenge (but see Diniz-Filho & Telles, 2006; Rissler *et al.*, 2006), particular in what refers to taxonomically complex groups of organisms, generally characterized by the presence of uniparental lineages and reticulate evolution (Ennos *et al.*, 2005).

The western Mediterranean is the ideal region to extend spatial conservation prioritization methodologies incorporating molecular data because, particularly for herptiles, there is already an extensive set of available molecular data at the subspecific level and an interesting evolutionary history dominated by successive cycles of fragmentation, expansion and subsequent admixture of populations. Consequently, in this region we can find multiple examples of species with exceptionally divergent lineages (e.g. Alexandrino *et al.*, 2007) and species complexes with incipient speciation (e.g. Perera *et al.*, 2007; Pinho *et al.*, 2007). The challenge is to integrate this dispersed and disparate data, collected in a multitude of geographical scales and using distinct molecular markers, and to find coherent spatial patterns of evolutionary processes that could be useful for conservation planning.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Map and description of the study area and Protected Areas network.

**Appendix S2** Species list and outputs of species distribution models.

**Appendix S3** List of species assigned to each biotic element.

**Appendix S4** Geographic distribution of the environmental categories identified with the principal components analysis.

**Appendix S5** Percentage of variance explained within the first and second axis of the principal components analysis within each biotic element and the overall study area, and the weight of each variable in each axis.

**Appendix S6** Comparison between biotic elements identified and phylogeographic patterns.

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**BIOSKETCH**

This study is part of **Silvia B. Carvalho's** PhD project, focused on biogeography and conservation of amphibians and reptiles in the western Mediterranean region. Her main research interests include the understanding of ecological, evolutionary and biogeographical patterns and processes to improve conservation planning strategies.

Author contributions: S.B.C., J.C.B., E.J.C. and H.P.P. conceived the ideas. J.C.B. and S.B.C. compiled the data and produced the database. S.B.C. analysed the data and led the writing with inputs from the remaining authors.

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