

GIS-based niche models identify environmental correlates sustaining a contact zone between three species of European vipers

F. Martínez-Freiria^{1*}, N. Sillero^{2,3}, M. Lizana¹ and J. C. Brito²

¹Departamento Biología Animal, Parasitología, Ecología, Edafología y Química Agrícola, Facultad de Biología, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain, ²CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Instituto de Ciências Agrárias de Vairão, R. Padre Armando Quintas, 4485-661 Vairão, Portugal, ³Departamento de Matemática Aplicada, Centro de Investigação em Ciências Geo-Espaciais (CICGE) da Universidade do Porto, R. Campo Alegre, 687, 4169-007 Porto, Portugal

ABSTRACT

The current range of European vipers is mostly parapatric but local-scale allopatric distribution is common and few cases of sympatry are known. In the High Course of Ebro River, northern Spain, there is a contact zone between *Vipera aspis*, *V. latastei*, and *V. seoanei*. Sympatry was detected between *aspis* and *latastei* and also specimens with intermediate morphological traits. Presence-data at a local scale (1 × 1 km) and ecological niche-based models manipulated in a GIS were used to (1) identify how environmental factors correlate with the distribution of the three vipers and with the location of the sympatry area, and (2) identify potential areas for viper occurrence and sympatry. Ensemble for casting with 10 Maximum Entropy models identified a mixture of topographical (altitude, slope), climatic (precipitation, evapotranspiration, and minimum and maximum temperature), and habitat factors (land cover) as predictors for viper occurrence. Similar predicted probabilities according to the variation of some environmental factors (indicating probable sympatry) were observed only for *aspis-latastei* and *aspis-seoanei*. In fact, areas of probable occurrence of vipers were generally allopatric but probable sympatry between vipers was identified for *aspis-latastei* in 76 UTM 1 × 1 km squares, for *aspis-seoanei* in 23 squares, and *latastei-seoanei* in two squares. Environmental factors correlate with the location of this contact zone by shaping the species range: some enhance spatial exclusion and constrain distribution to spatially non-overlapping ranges, while others allow contact between species. The distribution in the contact zone apparently results from the balance between the pressures exerted by the different environmental factors and in the sympatry area probably by interspecific competition. Further ecological and genetical data are needed to evaluate the dynamics of the probable hybrid zone. GIS and niche-modelling tools proved to be powerful tools to identify environmental factors sustaining the location of contact zones.

Keywords

Burgos, contact zone, ecological barriers, GIS, Maximum Entropy models, snakes, Viperidae.

*Correspondence: F. Martínez-Freiria, Departamento Biología Animal, Parasitología, Ecología, Edafología y Química Agrícola, Facultad de Biología, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain. E-mail: fmartinez_freiria@yahoo.es

INTRODUCTION

Allopatric speciation is perhaps the simplest and most frequent process of speciation (Brown & Lomolino, 1998). When populations are geographically isolated, lack of gene flow allows the development of regional genetic differences in response to either natural selection or genetic drift. In such cases, allopatric populations may become reproductively isolated, preventing gene flow to occur when populations meet in secondary contact (Schluter, 2001). During the Pleistocene ice ages of the Quaternary,

ancestors of many species experienced allopatric speciation processes in southern European refugia (Taberlet *et al.*, 1998). Ecological barriers or competing species later hampered the northwards expansion during warm interstages (Hewitt, 1996).

European vipers are an interesting group to study allopatric patterns of speciation. They constitute a monophyletic group differentiated from other Eurasian vipers in the early Miocene and evolved during the Quaternary dynamic scenario (Lenk *et al.*, 2001; Garrigues *et al.*, 2005). The expansion–contraction of ice shaped the distribution of different forms, the location of

population refugia, and established three allopatric clades: (1) *Pelias* group in northern Europe, including *Vipera berus* in almost all northern Europe and *V. seoanei* in northern Iberian peninsula; (2) *V. aspis* complex in western Mediterranean, including *V. aspis* in the Italian peninsula, southern France, and north-eastern Iberian peninsula, and *V. latastei* in the Maghreb and in almost all Iberian peninsula; and (3) *V. ammodytes* complex in the Balkans (Garrigues *et al.*, 2005).

The current range of European vipers is mostly parapatric and, at a broad scale (10 × 10 km grid cells or larger), several contact zones (i.e. areas where the range of species meet and may overlap or not) between distributions of the different species are known (Saint-Girons, 1980). Nevertheless, biogeographical studies conducted at local scale reported an allopatric distribution pattern (Duguay & Saint-Girons, 1978; Bea, 1985; Naulleau, 1986) and few cases of sympatry (i.e. areas where species overlap their geographical ranges) have been described; usually over areas of less than 1 km² (Saint-Girons, 1975; Monney, 1996). Environmental factors, such as altitude or precipitation, are correlated with the range of vipers in contact zones (Brito & Crespo, 2002; Guisan & Hofer, 2003), thus, vipers tend to select structurally different habitats and with distinct microclimatic conditions (Reinert, 1984a; Brito & Crespo, 2002). Thus, in most contact zones, their patterns of habitat selection linked with typical sedentary behaviour can promote lack of sympatry.

The high course of the Ebro River (hereafter High Ebro), northern Spain, is the only known contact zone between the three Iberian vipers, *V. aspis* (Linnaeus 1758), *V. latastei* (Boscá 1878), and *V. seoanei* (Lataste 1879). A recent survey of the region at a local scale (1 × 1 km grid cells), detected *V. aspis* in the north-eastern and central parts of the study area, *V. latastei* in the southern part and *V. seoanei* in the north-western part (Martínez-Freiria *et al.*, 2006). The typical allopatric distribution pattern at local scale was found for the phylogenetically distantly related pairs, *aspis-seoanei* and *latastei-seoanei* (Lenk *et al.*, 2001; Garrigues *et al.*, 2005). However, sympatry was detected for the closely related *aspis-latastei*, in an area of 8 km² along the middle course of the rivers Rudrón and Sedanillo (Fig. 1; Martínez-Freiria *et al.*, 2006). In fact, specimens with intermediate morphological characteristics (similar scale counts and colour pattern) were found in syntopic populations, suggesting that hybridization could occur between *V. aspis* and *V. latastei* (Martínez-Freiria *et al.*, 2006). However, the role of environmental factors on the dynamics of the High Ebro contact zone is unknown, particularly, how environment is related with the species range and acts as a potential ecological barrier to gene flow. Also, the multispecies scenario for the High Ebro is especially suitable to test the effects of environmental barriers in the location of contact zones.

There is a huge literature on contact zones in a multitude of taxa, and many important papers on the influence of environmental factors (e.g. Reinert, 1984a; Bishop *et al.*, 2002). However, few studies have addressed this relationship spatially, using Geographical Information System (GIS) tools (but see Anderson *et al.*, 2002; Swenson, 2006), and usually these studies performed over broad regions, such as continents (but see Brito & Crespo,

2002). In this context, the objectives of this study are to: (1) identify how environmental factors correlate with the distribution and the habitats selected by *V. aspis*, *V. latastei*, and *V. seoanei* in the High Ebro, and (2) identify potential areas for viper occurrence and sympatry at a local scale (1 × 1 km grid cells). Ecological niche-based models will be manipulated in a GIS to identify the environmental factors correlated with the location of the contact zone and sympatry area.

METHODS

Study area

The study area covers the 'Hoces del Alto Ebro y Rudrón' ('Natura 2000' network of European priority conservation areas) and adjacent mountains with a total area of 1200 km² (42°37.7' N to 42°58.7' N; 3°37.3' W to 3°58.5' W), located in the high course of the Ebro River, northern Spain (Fig. 1). The area consists of calcareous plateaus excavated by the Ebro River and its tributaries, Rudrón and Panero, forming canyons and steep valleys. Altitude ranges from 600 to 1250 m. Climate is subhumid Mediterranean with Central European tendency. The most representative bioclimatic stage is the Supra-Mediterranean but there are also elements of the Mountain stage of the Euro-Siberian region (for details see Martínez-Freiria *et al.*, 2006).

Environmental factors

The study area was divided in 1200 UTM 1 × 1 km squares and each square was characterized with a set of 7 uncorrelated ($r < 0.750$ in all cases) environmental factors (Table 1). Ecogeographical variables (hereafter EGV) known to affect the distribution of viperid snakes, including *V. aspis*, *V. latastei* and *V. seoanei*, were selected for analysis (Brito & Crespo, 2002; Guisan & Hofer, 2003; Santos *et al.*, 2006; N. Sillero & J.C. Brito, unpublished data). Four types of EGVs were considered: (1) topographical – altitude and slope derived from a digital elevation model (USGS, 2004); (2) climatic – a set of three global climate grids and one regional (evapotranspiration) derived from Worldclim version 1.4 (Hijmans *et al.*, 2005) and SIGA (2005), respectively; and (3) habitats – a land cover EGV composed of 14 months (1999–2000) satellite data acquired by the VEGETATION sensor on-board SPOT 4 satellite (GLC, 2003).

With the exception of land cover, EGVs were continuous and quantitative. The resolution of all EGVs was decreased to a grid cell size of 1 × 1 km, using the Aggregate function of ArcMap 9.0 GIS (ESRI, 2004), where each output grid cell of the new EGV contains the mean value of the input cells that were encompassed by the extent of the output cell.

Viper records

Between March 2004 and April 2007, visual encounter surveys and random road sampling based on the 1 × 1 km UTM grid were performed throughout the study area. Sampled squares were spatially distributed across the study area in order to cover

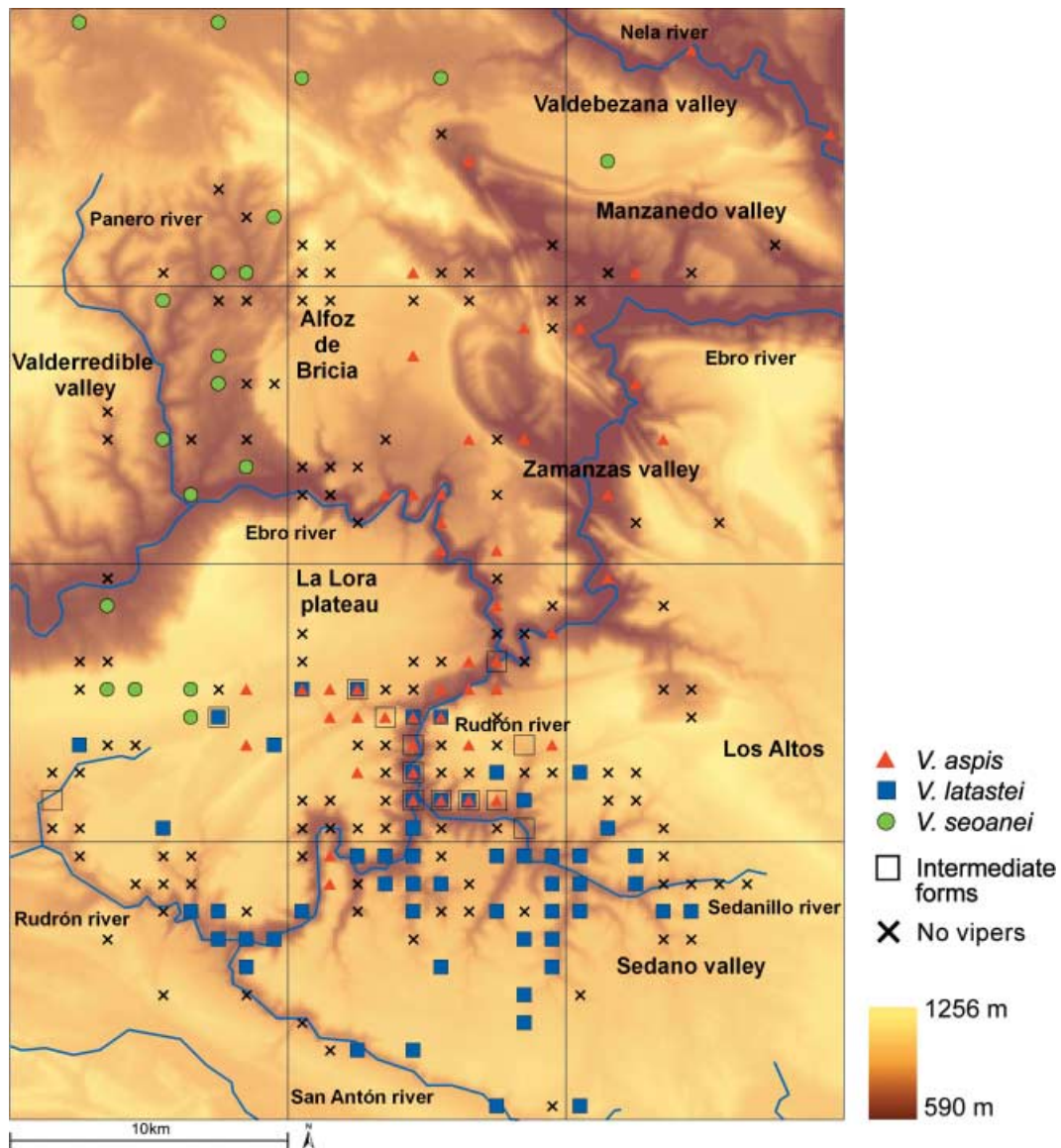


Figure 1 Presence records of *Vipera aspis* (Linnaeus 1758), *V. latastei* (Boscá 1878), and *V. seoanei* (Lataste 1879) and intermediate forms between *V. aspis* and *V. latastei* at a 1 × 1 km scale in the High Ebro, northern Spain. No vipers – sampled squares where vipers were not detected.

Table 1 Ecogeographical variables used for model the distribution of *Vipera aspis* (Linnaeus 1758), *V. latastei* (Boscá 1878), and *V. seoanei* (Lataste 1879) in the High Ebro, northern Spain.

Name	Units and classes	Source
Altitude	From 600 to 1250 m a.s.l.	USGS (2004)
Slope	From 0 to 45%	USGS (2004)
Precipitation	Annual average, from 698 to 908 mm year ⁻¹	Hijmans <i>et al.</i> (2005)
Evapotranspiration	Annual average, from 587 to 673 mm year ⁻¹	SIGA (2005)
Min temperature	Minimum temperature of the coldest month, from -1.5 to 2.1 °C	Hijmans <i>et al.</i> (2005)
Max temperature	Maximum temperature of the warmest month, from 22 to 24.5 °C	Hijmans <i>et al.</i> (2005)
Land cover	Six classes: broadleaved and deciduous forests, needle-leaved and evergreen forests, mixed leaf forests, shrubs, herbaceous, cultivated areas	GLC (2003)

the range of environmental variability (Fig. 1). In total, 256 squares were sampled, representing 21% of the study area.

Specimens were captured by hand and their geographical location (UTM coordinates; European-1950 datum) was recorded with a GPS. Specimens were classified as *V. aspis*, *V. latastei*, *V. seoanei*, or intermediate forms between *V. aspis* and *V. latastei* according to a combination of morphological characters: snout elevation, number of apical scales, shape of the dorsal stripe, and number of dorsal marks. Specimens were classified as intermediate when exhibiting contradictory or intermediate morphological traits (for details see Martínez-Freiria *et al.*, 2006). Viper records were inscribed in a georeferenced database and assigned to the corresponding 1 × 1 km UTM square.

Model building with maximum entropy methods

The spatial behaviour and activity patterns of vipers complicate the accurate determination of absences in a given square. The home range size is very small, the active season is short, and individuals remain mostly inside bushes through the year (Brito, 2003a,b). Therefore, to identify the environmental factors that are related to the distribution of each viper and locate areas of probable occurrence, the Maximum Entropy was used. This is a general-purpose machine learning method that uses presence-only occurrence data (Phillips *et al.*, 2004, 2006). Models were developed with Maxent 3.0.4 beta (<http://www.cs.princeton.edu/~schapire/maxent>), which is particularly well suited to noisy or sparse information, as is typical of species occurrence data, and capable of dealing with continuous and categorical EGVs at the same time (Phillips *et al.*, 2006). Also, comparative analyses of model performance revealed that Maximum Entropy models consistently outperform more established methods, including presence-only methods (Bioclim, Domain) and presence-absence methods (generalized additive models, generalized linear models), especially when samples sizes are low (Elith *et al.*, 2006; Hernandez *et al.*, 2006).

Essentially, Maximum Entropy models are based on a simple reasoning: when characterizing some unknown events with a statistical model, it should always be chosen the one that has maximum entropy, i.e. the one that produces the most uniform distribution but still infers as accurately as possible the observed data (e.g. maximize entropy for a given chi-squared value). Maxent estimates the range of species with the constraint that the expected value of each EGV (or its transform and/or interactions) should match its empirical average, i.e. the average value for a set of sample points taken from the species-target distribution (Phillips *et al.*, 2004, 2006). Maxent weights each EGV by a constant and the estimated probability distribution is exponential in the sum of the weighed features, divided by a scaling constant to ensure that the probability values range from 0.0 to 1.0 and sum to 1.0. The program starts with a uniform probability distribution (gain = 0) and iteratively alters one weight at a time to maximize the likelihood of the occurrence data set. The gain is a measure of the likelihood of the samples. The algorithm converges to the optimum probable distribution, thus, the gain can be interpreted as representing how much better the distribution fits

the sample points than the uniform distribution does (Phillips *et al.*, 2004, 2006).

From the 48, 54, and 19 UTM 1 × 1 km squares with presence records of *V. aspis*, *V. latastei*, and *V. seoanei*, respectively, 80% of the presence records were randomly selected by Maxent as training data and 20% reserved for model testing. Therefore, 39, 44, and 16 training records were used for *V. aspis*, *V. latastei*, and *V. seoanei*, respectively, and 9, 10, and 3 test records were used for *V. aspis*, *V. latastei*, and *V. seoanei*, respectively. The intermediate individuals were not used in the analysis as these vipers might be hybrids and bias the ecological models for the true forms.

Due to low sample size, 10 multivariate Maximum Entropy models were run with autofeatures and tested with receiver operated characteristics (ROC) plots. Since absence data were not available from the fieldwork, it was selected uniformly at random by Maxent from the background squares (Phillips *et al.*, 2006). The area under the curve (AUC) of the ROC plot was taken as a measure of the overall fit of the models (Liu *et al.*, 2005). The importance of each EGV for explaining the distribution of vipers was determined by: (1) jackknife analysis of the average gain with training and test data and also with AUC. EGVs were excluded in turn and a model created with the remaining variables; then a model was created using each individual variable, and in addition, a model was created using all variables; and (2) average percentage contribution of each EGV for the models. In each iteration of the training algorithm, the increase or decrease in regularized gain was added or subtracted, respectively, to the input of the corresponding variable, giving a heuristic estimate of EGV contribution for the model (Phillips *et al.*, 2006).

The relationship between occurrence of vipers and EGVs was determined by the visual examination of the profiles of response curves (see Austin, 1987). Similar profiles between two vipers for a given EGV were taken as an indication of identical relationships between the occurrence of these species and the range of variation of the EGV. This would indicate also the possible occurrence of sympatry within the range of values of the EGV equally selected by both species. A distinct profile of a viper in relation to the others vipers was taken as an indication of a divergent relationship and possible exclusion of that species within the range of values of the EGV-selected exclusively. Response curves represent the exponent changes, i.e. predicted suitability, as each EGV varies by keeping all other variables at their average sample value (Phillips *et al.*, 2004). Therefore, univariate models were developed using each EGV solely in order to avoid possible biases by other correlated EGVs.

Probability of occurrence maps

The Maximum Entropy model classifies squares with a continuous value of probability of occurrence between 0 and 1. Traditionally, a threshold is estimated to reclassify probability of occurrence maps into areas of probable presence/absence (e.g. Brito & Crespo, 2002). However, the issue of selecting an optimized threshold with Maxent and selecting the 'best' model from a model ensemble is rather subjective (Phillips *et al.*, 2004; Phillips *et al.*, 2006). Therefore, instead of picking the 'best' model from an ensemble,

a more promising approach is to explore the resulting range of projections, a procedure known as ensemble forecasting (Araújo & New, 2007). With this method, the 10 raw models for each species were added to generate probability distribution functions, i.e. a synthetic combined forecast of all models showing the likelihood of species presence.

To identify areas of probable sympatry between pairs of vipers, the consensus forecasting was used, where a measure of the central tendency (the mean) was calculated for the ensemble of 10 forecasts. The rationale behind consensus forecasts is that, in averaging several models, main patterns of distribution emerge from the probably biased individual models (Araújo & New, 2007). Therefore, the synthetic maps for each viper were overlaid in the ArcMap GIS and reclassified according to a consensus threshold of a minimum of five forecasts for one species and 10 for the other species to a maximum of 10 forecasts for each species.

RESULTS

The Maximum Entropy models identified a set of topographical and climatic EGVs explaining the distribution of vipers (Table 2, Fig. 2). The distribution of *V. aspis* is related to altitude, slope, precipitation, evapotranspiration, and land cover; *V. latastei* is related to slope, precipitation, evapotranspiration, and maximum temperature; and *V. seoanei* is related to altitude, slope, precipitation, evapotranspiration, and minimum temperature. The distribution of vipers is influenced by common EGVs, such as altitude, slope, precipitation, and evapotranspiration (Table 2, Fig. 2).

The profiles of the response curves for the EGVs related to the distribution of two or more species revealed similar patterns for pairs of vipers (Fig. 3): (1) *V. aspis* and *V. latastei* occur more frequently in steeper areas; (2) *V. aspis* and *V. seoanei* occur in areas with average precipitation above 750 mm year⁻¹ and evapotranspiration between 590 and 620 mm year⁻¹; and (3) *V. latastei* and *V. seoanei* occur in areas with distinct environmental characteristics (Fig. 3). In comparison with the remaining vipers, specific patterns were observed: (1) *V. aspis* occurs mostly in areas with evapotranspiration above 620 mm and less frequently above 1000 m of altitude; (2) *V. latastei* occurs less frequently in

areas with average precipitation above 750 mm year⁻¹ and evapotranspiration also above 660 mm; and (3) *V. seoanei* occurs mostly in flat areas and also above 1000 m of altitude (Fig. 3).

The ROC plots for the training data set exhibited average AUCs above 0.910 for all species, whereas average AUCs for test data set were lower, but all above 0.780 (Table 2).

Areas of probable occurrence of vipers were identified for: (1) *V. aspis* in the north-eastern and central parts of the study area, along the Ebro, middle and lower Rudrón, and lower Sedanillo rivers; (2) *V. latastei* in the southern part, along the Rudrón and Sedanillo; and (3) *V. seoanei* in the northern and central-western parts, along the Panero river and Zamanzas valley, and in isolated patches of mountain peaks (Fig. 4). Areas of potential sympatry between vipers were identified for (1) *V. aspis* and *V. latastei* in 76 UTM 1 × 1 km squares located in the central part of the study area; (2) *V. aspis* and *V. seoanei* in 23 squares scattered through the central part; and (3) *V. latastei* and *V. seoanei* in two squares located in the western-central part (Fig. 5).

DISCUSSION

Environmental factors are correlated with the dynamic range scenario of vipers in the High Ebro. Some factors enhance spatial exclusion for some species pairs while favouring sympatry for others. For instance, precipitation hampers the contact of *V. latastei* with *V. aspis* and *V. seoanei*, and facilitates sympatry among the latter vipers. The same relation occurs with slope but for a different pair of species: contact of *V. seoanei* with *V. aspis* and *V. latastei* is hindered above 10% of slope but promoted between the latter vipers. More interestingly, altitude and evapotranspiration support sympatry between *V. aspis* and *V. seoanei* below 1000 m and below 620 mm, respectively, but favour spatial exclusion above these thresholds. Thus, some factors constrain the distribution of vipers in the High Ebro to spatially non-overlapping ranges, acting as ecological barriers to gene flow, while others facilitate sympatry allowing spatial coexistence and even hybridization.

Studies examining the qualitative importance of environmental factors in contact zones between European vipers (but see Brito

Table 2 Average (minimum–maximum) percentage contribution of each variable and average (minimum–maximum) training and test area under the curve (AUC) for the 10 Maximum Entropy models for *Vipera aspis*, *V. latastei*, and *V. seoanei* in the High Ebro, northern Spain.

	<i>V. aspis</i>	<i>V. latastei</i>	<i>V. seoanei</i>
Altitude	5.76 (3.0–8.5)	2.56 (1.1–4.4)	7.67 (4.0–14.5)
Precipitation	12.52 (10.0–19.7)	54.80 (50.9–57.5)	20.28 (13.4–27.3)
Evapotranspiration	44.71 (40.5–48.9)	16.53 (14.8–18.8)	17.95 (12.5–24.7)
Land Cover	14.55 (10.9–17.6)	0.89 (0.3–1.6)	2.15 (0.4–5.4)
Slope	9.58 (4.3–16.1)	9.65 (5.7–16.0)	16.62 (11.2–20.0)
Maximum temperature	4.85 (2.7–8.9)	7.97 (1.4–14.2)	6.25 (3.4–10.0)
Minimum temperature	7.99 (5.0–10.5)	7.60 (5.7–9.9)	29.10 (21.8–41.7)
Training AUC	0.915 (0.903–0.927)	0.951 (0.943–0.960)	0.961 (0.946–0.970)
Test AUC	0.782 (0.707–0.865)	0.873 (0.809–0.935)	0.780 (0.708–0.923)

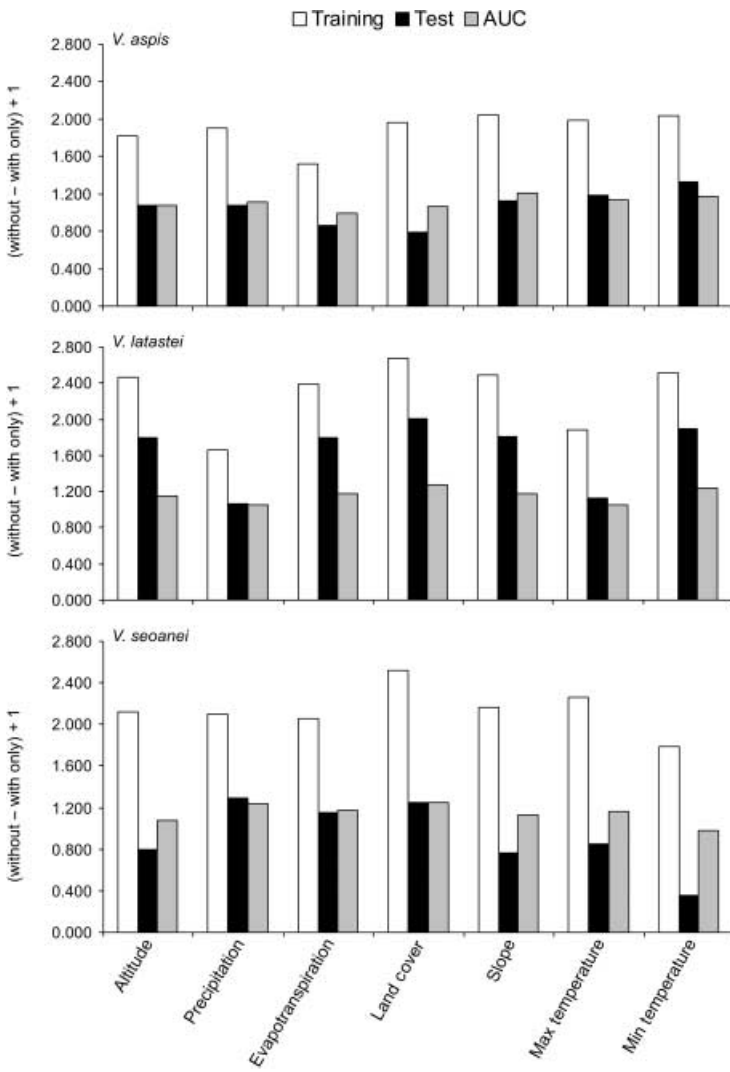


Figure 2 Jackknife results for 10 Maximum Entropy models of the distribution of *Vipera aspis*, *V. latastei*, and *V. seoanei* in the High Ebro, northern Spain. For each variable it is presented the difference in average gain and area under the curve (AUC) on test data between models built without a given variable and models built with only that variable. Variables with smaller differences both in gain and in AUC are the most related to the distribution of each species.

& Crespo, 2002) revealed distinct habitat selection patterns according to phylogenetical relationships. Species of Mediterranean origin, such as *V. aspis*, *V. latastei*, or *V. ammodytes*, tend to occur in warmer and dryer habitats located at lower altitudes, in comparison with species of Euro-Siberian origin, such as *V. berus*, *V. seoanei*, or *V. ursinii*, which are usually found in fresher and more humid habitats located at higher altitudes (Saint-Girons, 1975, 1980; Duguay *et al.*, 1979; Bea, 1985; Monney, 1996). In the High Ebro, *V. latastei* and *V. seoanei* replicate these patterns of habitat selection, but *V. aspis* exhibits an intermediate selection pattern between these two vipers, occurring in warmer (high levels of slope and evapotranspiration) and more humid (high levels of precipitation) areas located at lower altitudes. Distinct habitat selection patterns are usually related to species physiological traits as a response to environmental variability. For instance, precipitation and temperature (both minimum and maximum) affect the duration of the active season of vipers, and, consequently, feeding and growth rates, gestation period, and reproductive frequency (Saint-Girons, 1975, 1980). Data on thermoregulation and reproductive traits are needed to

understand how environmental factors exert selective pressures on both vipers at local scale.

The general allopatric distribution pattern of vipers in the High Ebro is similar to other contact zones in Europe. Only a few cases of sympatry have been reported, mostly between *V. aspis* and *V. berus*: a narrow band of 1–2 km in the Atlantic-Loire region, west of France (Saint-Girons, 1975), and in a 70-ha area in the Pre-Alps, west of Switzerland (Monney, 1996). The distribution pattern in the High Ebro also replicates the evolutionary relationships among European vipers (Lenk *et al.*, 2001; Garrigues *et al.*, 2005). No sympatry was found between the phylogenetically distantly related viper, *V. seoanei*, and *V. aspis* or *V. latastei* (Martínez-Freiria *et al.*, 2006), and areas of potential sympatry identified in this study between *V. seoanei* and the other two vipers were small (less than 23 km²). Thus, ecological barriers among non-sibling vipers appear to be very strong. For instance, in contact zones between *V. latastei* and *V. seoanei* in the Portuguese Gerês mountains or between *V. aspis* and *V. seoanei* in the Spanish Bask country, no sympatry was detected, and differential habitat selection patterns, even opposite, were suggested as responsible

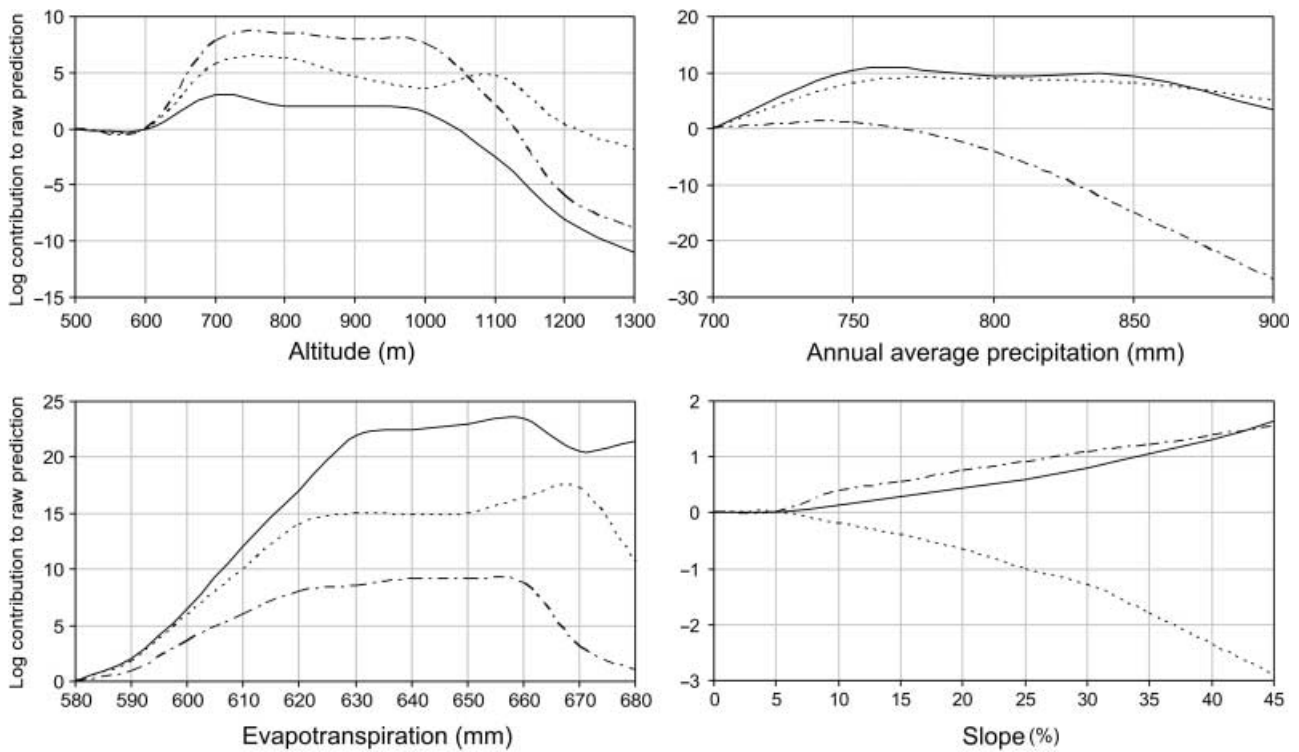


Figure 3 Response curves for the environmental factors related to of two or more vipers in the High Ebro, northern Spain. *Vipera aspis* (—), *V. latastei* (-.-.-), and *V. seoanei* (..).

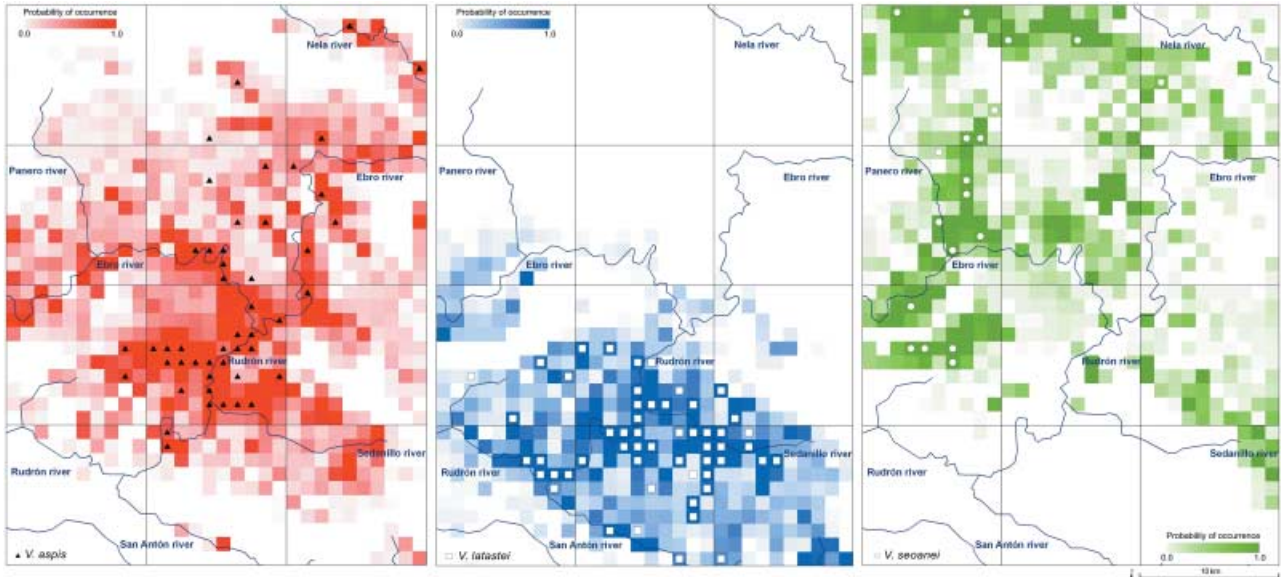


Figure 4 Probability density function with the likelihood of occurrence of *Vipera aspis*, *V. latastei*, and *V. seoanei* in the High Ebro, northern Spain, at a 1 × 1 km scale estimated by the ensemble of 10 Maximum Entropy models.

for the allopatric distribution at local scale (Bea, 1985; Brito & Crespo, 2002).

Sympatry between the phylogenetically closely related *V. aspis* and *V. latastei* was found in the High Ebro over a relatively large area (8 km², Martínez-Freiria *et al.*, 2006), and the current study

identified a potential sympatry area of 76 km². Intermediate vipers ($n = 13$ squares) were mostly found in this potential area ($n = 9$ squares) or in contiguous localities ($n = 3$ squares) (Fig. 5). Specimens of intermediate and true forms were found together during the mating season (Martínez-Freiria *et al.*,

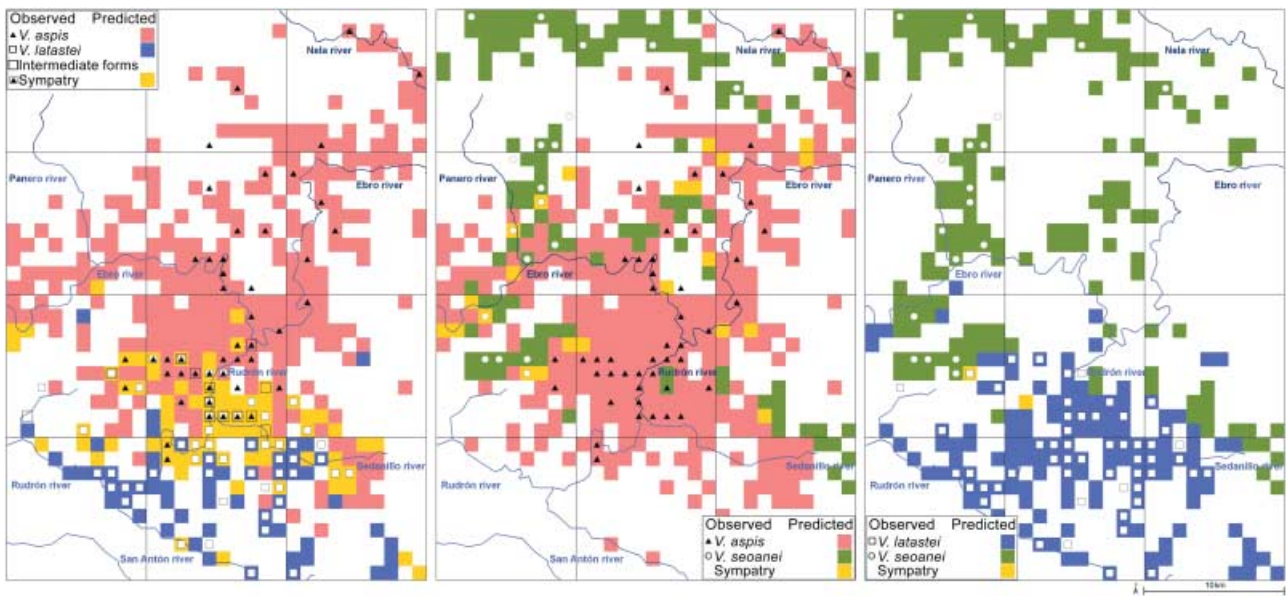


Figure 5 Consensus forecast showing the area where at least half (the mean) of the 10 Maximum Entropy models predict the presence of vipers in the High Ebro, northern Spain, at a 1 × 1 km scale. Areas of probable sympatry (*Vipera aspis*–*V. latastei*, *V. aspis*–*V. seoanei*, and *V. latastei*–*V. seoanei*) were estimated by the ensemble of 15–20 forecasts, i.e. sympatry areas ranged from a minimum of five forecasts for one species and 10 for the other to a maximum of 10 forecasts for each species.

2006), suggesting the occurrence of hybridization. Usually, habitat partition avoids interspecific competition and ultimately reproductive isolation in sympatric vipers (Luiselli, 2006). However, the ecological-niche model indicates that habitats along Rudrón and Sedanillo river valleys present environmental traits favouring population mixture. In these areas, vipers are probably competing for prey, thermoregulation spots, hibernation shelters, and probably mates, as recorded for other sympatric snakes (Reinert, 1984b; Beck, 1995; Luiselli *et al.*, 2002, 2006). Thus, the distribution of vipers in the contact zone apparently results from the balance between the pressures exerted by different environmental factors and in the sympatry area probably by interspecific competition. Reproductive isolation mechanisms, including chemical signalling and zygote viability, and fitness of probable hybrids should be extremely important in the dynamics of this contact zone, as suggested for other sympatric taxa (Shine *et al.*, 2002; Barbosa *et al.*, 2006).

Ecological niche-based models developed in a spatial context identified environmental factors correlated with each viper range in the High Ebro contact zone. The combination of these models with GIS has been used successfully to analyse hybrid zone dynamics in other taxonomic groups (Swenson & Howard, 2005; Swenson, 2006). Therefore, such models would be useful to identify other potential sympatry areas in southern Pyrenees, where *V. aspis* and *V. latastei* also exhibit a wide contact zone. Although *V. aspis* tends to select fresh and humid areas in north-facing slopes, whereas *V. latastei* selects rocky and dry areas in south-facing slopes (Duguay *et al.*, 1979), intermediate vipers were found, suggesting also the occurrence of hybrid zones. Moreover, GIS-assisted ecological models could be used to clear evolutionary relationships through the identification of potential

hybrid zones among sibling and sympatric species, such as in African and Iberian *Podarcis* lizards (e.g. Pinho *et al.*, 2006).

In summary, environmental factors play an important role in the dynamics of this contact zone affecting the species range. Some factors seem to promote spatial exclusion whereas others endorse the spatial overlap and sympatry. Ecological niche-based models identified the environmental factors that are correlated with the location of the sympatry area and probably act as ecological barriers to interspecific gene flow. Further ecological and genetical data are being collected to evaluate the dynamics of the probable hybrid zone and to establish optimize conservation measures.

ACKNOWLEDGEMENTS

This study was partially supported by project POCTI/BIA-BDE/55596/2004 from Fundação para a Ciência e Tecnologia (FCT, Portugal). FMF was supported by PhD grant (AP2003-2633) from Ministerio de Educación, Cultura y Deporte (Spain) and JCB and NS were supported by post-doctoral grants (SFRH/BPD/26699/2006 and SFRH/BPD/26666/2006, respectively) from FCT. Authors acknowledge Servicio Transfronterizo de la Universidad de Salamanca and ‘Asociación Sociocultural Hoces del Alto Ebro y Rudrón’ (Burgos, Spain). X Santos, JM Pleguezuelos, MA Carretero, and three referees improved earlier versions of the manuscript.

REFERENCES

Anderson, R.P., Gómez-Laverde, M. & Peterson, T. (2002) Geographical distributions of spiny pocket mice in South America:

- insights from predictive models. *Global Ecology and Biogeography*, **11**, 131–141.
- Araújo, M.B. & New, N. (2007) Ensemble forecasting of species distributions. *Trends in Ecology & Evolution*, **22**, 42–47.
- Austin, M.P. (1987) Models for the analysis of species' response to environmental gradients. *Vegetatio*, **69**, 35–45.
- Barbosa, D., Font, E., Desfilis, E. & Carretero, M.A. (2006) Chemically-mediated species recognition in closely related *Podarcis* wall lizards. *Journal of Chemical Ecology*, **32**, 1587–1598.
- Bea, A. (1985) La repartición de las víboras *Vipera aspis* (Linnaeus, 1758) y *Vipera seoanei* (Lataste, 1879), en el País Vasco. *Ciencias Naturales*, **2**, 7–20.
- Beck, D.D. (1995) Ecology and energetics of three sympatric rattlesnakes species in the Sonoran Desert. *Journal of Herpetology*, **29**, 211–223.
- Bishop, A.A., Hoback, W.W., Albrecht, M. & Skinner, K.M. (2002) A comparison of an ecological model and GIS spatial analysis to describe niche partitioning amongst Carrion Beetles in Nebraska. *Transactions in GIS*, **6**, 457–470.
- Brito, J.C. (2003a) Seasonal variation in movements, home range and habitat use by male *Vipera latastei* in northern Portugal. *Journal of Herpetology*, **37**, 155–160.
- Brito, J.C. (2003b) Seasonal and daily activity patterns of *Vipera latastei* in northern Portugal. *Amphibia-Reptilia*, **24**, 497–508.
- Brito, J.C. & Crespo, E.G. (2002) Distributional analysis of two vipers (*Vipera latastei* and *V. seoanei*) in a potential area of sympatry in the North-western Iberian Peninsula. *Biology of the Vipers* (ed. by G.W. Schuett, M. Höggren, M.E. Douglas and H.W. Greene), pp. 129–138. Eagle Mountain Publishing, Eagle Mountain, Utah.
- Brown, J.H. & Lomolino, M.V. (1998) *Biogeography*. Sinauer Associates Inc., Sunderland, Massachusetts.
- Duguy, R., Martínez-Rica, J.P. & Saint-Girons, H. (1979) La répartition des vipères dans les Pyrénées et les régions voisines du nord de l'Espagne. *Bulletin de la Société d'Histoire Naturelle de Toulouse*, **115**, 359–377.
- Duguy, R. & Saint-Girons, H. (1978) La répartition des vipères et de quelques autres reptiles sur le plateau de Millevaches (Limousin). *Annales de la Société Des Sciences Naturelles Charente-Maritime*, **6**, 351–354.
- Elith, J., Graham, C.H., Anderson, R.P., Dudyk, M., Freer, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A., Li, J., Lohmann, L.G., Loiselle, B.A., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., McOverton, J., Peterson, A.T., Phillips, S., Wisz, M.S. & Zimmermann, N.E. (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129–151.
- Environmental Systems Research Institute, Inc. (ESRI) (2004) *Arcmap 9.0*. ESRI, Redlands, California.
- Garrigues, T., Dauga, C., Ferquel, E., Choumet, V. & Failloux, A.-B. (2005) Molecular phylogeny of *Vipera* Laurenti, 1768 and the related genera *Macrovipera* (Reuss, 1927) and *Daboia* (Gray, 1842), with comments about neurotoxic *Vipera aspis aspis* populations. *Molecular Phylogenetics and Evolution*, **35**, 35–47.
- GLC (2003) *The global land cover for the year 2000*. <http://www-gvm.jrc.it/glc2000/defaultGLC2000.htm>.
- Guisan, A. & Hofer, U. (2003) Predicting reptile distributions at mesoscale: relation to climate and topography. *Journal of Biogeography*, **30**, 1233–1243.
- Hernandez, P.A., Graham, C.H., Master, L.L. & Albert, D.L. (2006) The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, **29**, 773–785.
- Hewitt, G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978. <http://www.worldclim.org/>.
- Lenk, P., Kalyabina, S., Wink, M. & Joger, U. (2001) Evolutionary relationships among the true vipers (Reptilia: Viperidae) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, **19**, 94–104.
- Liu, C., Berry, P.M., Dawson, T.P. & Pearson, R.G. (2005) Selecting thresholds of occurrence in the prediction of species distributions. *Ecography*, **28**, 385–393.
- Luiselli, L.M. (2006) Resource partitioning and interspecific competition in snakes: the search for general geographical and guild patterns. *Oikos*, **114**, 193–211.
- Luiselli, L.M., Angelici, F.M. & Akani, G. (2002) Comparative feeding strategies and dietary plasticity of the sympatric cobras *Naja melanoleuca* and *Naja nigricollis* in three diverging Afrotropical habitats. *Canadian Journal of Zoology*, **80**, 55–63.
- Martínez-Freiría, F., Brito, J.C. & Lizana, M. (2006) Intermediate forms and syntopy among vipers (*V. aspis* and *V. latastei*) in Northern Iberian Peninsula. *Herpetological Bulletin*, **97**, 14–18.
- Monney, J.-C. (1996) *Biologie comparée de Vipera aspis L. et de Vipera berus L. (Reptilia, Ophidia, Viperidae) dans une station des Préalpes Bernoises*. PhD Thesis. Institute of Zoology, Faculty of Sciences, University of Neuchatel, Neuchatel, Switzerland.
- Naulleau, G. (1986) Répartition de *Vipera aspis* et de *Vipera berus* (Reptilia, Viperidae), dans l'ouest de la France (Loire-Atlantique). *Bulletin de la Société Herpetologique de France*, **39**, 16–19.
- Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.
- Phillips, S.J., Dudík, M. & Schapire, R.E. (2004) A Maximum Entropy approach to species distribution modeling. *Proceedings of the 21st International Conference on Machine Learning*, pp. 655–662. ACM Press, New York.
- Pinho, C., Ferrand, N. & Harris, D.J. (2006) Reexamination of the Iberian and North African *Podarcis* (Squamata: Lacertidae) phylogeny based on increased mitochondrial DNA sequencing. *Molecular Phylogenetics and Evolution*, **38**, 266–273.
- Reinert, H.K. (1984a) Habitat separation between sympatric snake populations. *Ecology*, **65**, 478–486.
- Reinert, H.K. (1984b) Habitat variation within sympatric snake populations. *Ecology*, **65**, 1673–1682.

- Saint-Girons, H. (1975) Coexistence de *Vipera aspis* et de *Vipera berus* en Loire-Atlantique: un probleme de competition interspecific. *La Terre et la Vie*, **29**, 590–613.
- Saint-Girons, H. (1980) Biogéographie et évolution des vipères européennes. *Comptes Rendus de la Société de Biogéographie*, **496**, 146–172.
- Santos, X., Brito, J.C., Sillero, N., Pleguezuelos, J.M., Llorente, G.A., Fahd, S. & Parellada, X. (2006) Inferring habitat-suitability areas with ecological modelling techniques and GIS: a contribution to assess the conservation status of *Vipera latastei*. *Biological Conservation*, **130**, 416–425.
- Schluter, D. (2001) Ecology and the origin of species. *Trends in Ecology & Evolution*, **16**, 372–380.
- Shine, R., Reed, R.N., Shetty, S., Lemaster, M.P. & Mason, R.T. (2002) Reproductive isolating mechanisms between two sympatric sibling species of sea snakes. *Evolution*, **56**, 1655–1662.
- Servicio de Información Geográfico Agrario (SIGA) (2005) Sistema de Información Geográfica de Datos Agrarios (SIGA). Ministerio de Agricultura, Pesca y Alimentación, Spain. <http://www.mapya.es/es/sig/pags/siga/intro.htm>.
- Swenson, N.G. (2006) GIS-based niche models reveal unifying climatic mechanisms that maintain the location of avian hybrid zones in a North American suture zone. *Journal of Evolutionary Biology*, **19**, 717–725.
- Swenson, N.G. & Howard, D.J. (2005) Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. *The American Naturalist*, **166**, 581–591.
- Taberlet, P., Fumaggali, L., Wust-Saucy, A.-G. & Cosson, J.F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.
- United States Geological Survey (USGS) (2004) *Shuttle radar topography mission (SRTM): mapping the world in 3 dimensions*. USGS, Reston, Virginia. <http://srtm.usgs.gov/index.php>.