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## Geographical patterns of morphological variation and environmental correlates in contact zones: a multi-scale approach using two Mediterranean vipers

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### Abstract

Adaptation to similar selective pressures can explain morphological convergence between closely related species in contact zones. Geostatistics and Geographical Information System were used to identify multi-scale patterns of morphological variability and test the hypothesis of morphological convergence due to local environmental pressures in the contact areas between *Vipera aspis* and *Vipera latastei* in the Iberian Peninsula. Nine morphological traits from 630 and 362 vipers at regional and local scale, respectively, were interpolated by Kriging to generate surfaces of morphological variation. Kriging is a geostatistical algorithm that allows investigating the spatial structure of data with statistical models. At both scales, a convergent north–south pattern in morphological variability was observed and the contact areas were identified as integration zones where intermediate vipers are found. Significant correlations were found between surfaces of univariate and multivariate traits, with precipitation and temperature seasonality. Thus, several morphological traits were apparently under local environmental selection. Nevertheless, the influence of biotic pressures and gene flow on morphological convergence of vipers in contact zones deserves further study.

**Key words:** Adaptation – environmental pressures – geographical variation – geostatistics – GIS – hybridization – Iberian Peninsula – interpolation – morphology – vipers

### Introduction

Natural selection is the evolutionary process by which heritable favourable variations due to particular environments are preserved and become more common in successive generations in a population (Darwin 1859). In species with wide distributions, selective pressures can change throughout their ranges and different phenotypes can be favoured as the most efficient forms to exploit local conditions (West-Eberhard 2003). In fact, geographical variation in morphological traits is a ubiquitous phenomenon in organisms and only a very restricted number of species do not show it (Thorpe 1987). There are many examples of how animals have adapted accordingly to selective pressures (Stearns and Hoekstra 2000; Pough et al. 2005). As ectotherms, reptiles are suitable models to explore morphological adaptability to local conditions due to their dependence on climatic variables such as temperature and rainfall (Pough 1980). For instance, temperature can act as a selective factor that leads to the development of different mechanisms along environmental gradients to maintain heat, such as selection for body size in reptiles (Bergmann's rule, Ashton and Feldman 2003), or to prevent water loss, such as increasing–decreasing scale numbers (Malhotra and Thorpe 1997; Sanders et al. 2004). Also predation can lead to mimicry adaptation where different variations in the dorsal colour pattern in agreement with the vegetation cover of the substrate are presumed to reduce predation risk over nearby populations (King 1997).

Contact zones between closely related species are very interesting for studying geographical variations in morphology. Usually they correspond to areas of secondary contact between allopatric species (Brown and Lomolino 1998) where species may experience interspecific gene flow as a result of hybridization (Babik et al. 2005; Beetles et al. 2005) and in many cases exhibit morphological convergence (Arif et al.

2007; Gifford 2008). Two main hypotheses can be drawn to explain morphological convergence between closely related species in contact zones: adaptation to similar selective pressures, including biotic and abiotic factors, or hybridization processes with genetic exchange between species. The former can be tested by experimental analyses (e.g. Blanckenhorn and Demont 2004) or alternatively by examining correlations between selective pressures and morphological variations (e.g. Báez and Brown 1997), whereas the latter can be tested by molecular studies.

In the Iberian Peninsula there are three parapatric viper species, two sibling Mediterranean, *Vipera aspis* and *Vipera latastei*, and one phylogenetically distant Euro-Siberian, *Vipera seoanei* (Lenk et al. 2001; Garrigues et al. 2005), which have several contact zones along their distribution areas (Fig. 1a). An allopatric distribution pattern at the regional scale (10 × 10 km cells) is found for the phylogenetically distantly related pairs, *aspis-seoanei* and *latastei-seoanei*, but sympatry was suggested for the closely related *aspis-latastei* in six areas of the Iberian Peninsula (Fig. 1b): one in northern Iberian System, four in southern Pyrenees (Sierra de la Peña, Sobrarbe, Pallars, Moianés) and one in the high course of the Ebro River (Pleguezuelos et al. 2002). No data are available for the Iberian System and the southern Pyrenees are poorly known with the exception of Sierra de la Peña, where a wide contact zone with populations in apparent sympatry was reported along a 10 km west–east band (Duguy et al. 1979). In the high course of the Ebro River (hereafter High Ebro), sympatry was observed in an area of 8 km<sup>2</sup> (Fig. 1c) along the middle course of the rivers Rudrón and Sedanillo (Martínez-Freiría et al. 2006) and ecological niche modelling techniques identified an area of 76 km<sup>2</sup> where *V. aspis* and *V. latastei* could potentially be found together (Martínez-Freiría et al. 2008). Interestingly, specimens with intermediate morpholog-

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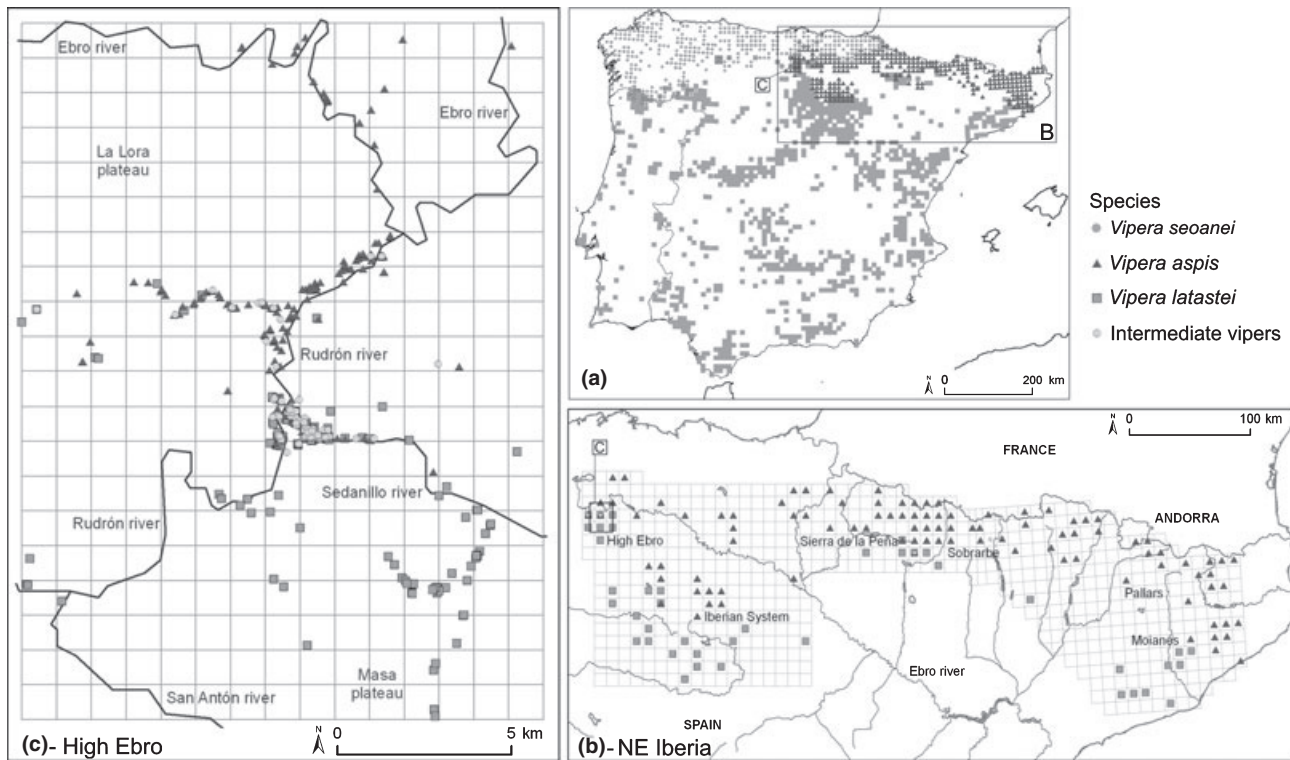


Fig. 1. (a) Distribution of Iberian vipers and geographical location of the study areas in the Iberian Peninsula (adapted from Pleguezuelos et al. 2002; Brito et al. 2006). (b) Study area of NE Iberia with the location of  $10 \times 10$  UTM cells with specimens of *Vipera aspis*, *Vipera latastei* and intermediate forms, and geographical location of the contact zones between *V. aspis* and *V. latastei*. (c) Study area of High Ebro with the GPS locations of specimens of *V. aspis*, *V. latastei* and intermediate forms

ical characteristics (similar scale counts and colour pattern) were found in Sierra de la Peña and High Ebro (Duguy et al. 1979; Martínez-Freiría et al. 2006). Furthermore, preliminary analyses of microsatellite variation in 195 specimens from High Ebro revealed the existence of gene flow among both species and that morphologically intermediate vipers correspond mostly to hybrids (authors, unpublished data).

Geographical variation in morphology has taken much importance since the latest decades of the past century (Thorpe 1987 and references therein). Traditionally, multivariate analysis using discrete tools such as principal component analysis (PCA) or canonical variate analysis (CVA) are used to identify geographical patterns of variation in morphological traits allowing the proposal of ecological and evolutionary hypotheses (e.g. Thorpe 1984; Wüster and Thorpe 1989). Later, these tools were combined with interpolation algorithms allowing the production of continuous surfaces of morphological variation (e.g. Thorpe and Báez 1987; Báez and Brown 1997) and therefore, enlightening geostatistical approaches as powerful tools for analysing geographical patterns of variation in morphological characters. Recently, the combination of geostatistics with Geographical Information Systems (GIS) has resurrected the importance of geography in morphology (Kidd and Ritchie 2000; Swenson 2008). In fact, GIS are useful tools to analyse geographical patterns of morphological and genetical variation (Cesaroni et al. 1997; Kidd and Ritchie 2000; Brito et al. in press), the dynamics of gene flow (Spear et al. 2005) and the identification of environmental factors related to species distribution in contact zones (Swenson 2006, 2008; Martínez-Freiría et al. 2008).

Knowing that gene flow exists among *V. aspis* and *V. latastei* (authors, unpublished data), in this work we tested the hypothesis of morphological convergence between both species due to an adaptation to similar local environmental pressures. Geostatistics and GIS tools were used to derive spatially explicit maps of the patterns of geographical variation in external morphological traits along the contact area. Adaptation to local environmental pressures were tested by exploring spatial correlations between morphological patterns and abiotic environmental pressures. Intermediate vipers were not used in the analysis because they are mostly hybrids, thus obscuring patterns of morphological variation and their interaction with the environment. Analyses were conducted at two complementary scales in order to analyse patterns and correlations independently of scale: regional, including all known contact areas in north-eastern Iberia, and local, focusing the analysis on the sympatry area of High Ebro. Therefore, the aims of this study focused on the following questions: (1) are there patterns of spatial variation in particular morphological traits for *V. latastei* and *V. aspis* in contact zones? (2) Can multivariate patterns of spatial variation in morphology be identified? (3) Are there regions of sharp transition in morphological variability? (4) Are morphological trait variations correlated with abiotic factors?

## Materials and Methods

### Study areas

At the regional scale, the study area covers 64 500 km<sup>2</sup> and it is located in the north-eastern of Iberian Peninsula, and includes all known contact zones between *V. aspis* and *V. latastei* (Fig. 1). It is limited to

the north by the Pyrenees chain and Bask Mountains and to the south by the Ebro Valley, to the west by the High Ebro and the Iberian System and to the east by the Mediterranean Sea. Altitude ranges from 0 to 3404 m a.s.l., average annual precipitation from 438 to 1759 mm year<sup>-1</sup> and average temperature from 19.9 to 30.0°C (Hijmans et al. 2005). The area coincides with the transition between the Euro-Siberian and Mediterranean regions.

At the local scale, the study area of High Ebro covers 300 km<sup>2</sup> and includes the sympatry area between *V. aspis* and *V. latastei* on the high course of the Ebro River (Fig. 1). This area also matches with the transition between Euro-Siberian and Mediterranean regions, consisting of flat plateaus with deep valleys excavated by the Ebro River and its tributaries, Rudrón and Sedanillo rivers. Altitude ranges from 621 to 1150 m a.s.l., average annual precipitation from 697 to 829 mm year<sup>-1</sup> and average annual temperature from 22.2 to 24.8°C (Hijmans et al. 2005).

### The species

Case species are terrestrial viviparous old world vipers (Serpentes, Viperinae). *Vipera aspis* (Linnaeus, 1758) occurs in central-western Europe whereas *V. latastei* Boscá, 1878 is restricted to the Iberian Peninsula, but with isolated populations in the Maghreb (Pleguezuelos et al. 2002). Morphologically, both species differ in the elevation of snout, number of apical scales and dorsal coloration pattern (see Morphological variation below). Habitat requirements are generally different between species but can overlap, allowing sympatry in contact zones such as High Ebro: *V. aspis* occurring in steeper areas at lower altitude, with moderate levels of precipitation, high levels of evapotranspiration and needle-leaved and evergreen forests whereas *V. latastei* also occurring in steeper but warmer areas, with low levels of precipitation and moderate levels of evapotranspiration (Martínez-Freiria et al. 2008). In High Ebro, both species select rocky areas with bushes during most of the year, but during summer, *V. aspis* selects warmer micro-habitats than *V. latastei* (authors, unpublished data). Home-range size is small in both species throughout most of the year, but males increase it during the mating season (Moser et al. 1984; Brito 2003a). Mating can occur in spring or autumn and births take place in middle or late summer (Lourdais et al. 2004; Pleguezuelos et al. 2007). Both vipers are ambush predators, taking mostly lizards as juveniles and micro-mammals as adults (Bea et al. 1992; Santos et al. 2007).

### The data

A total of 728 vipers, 276 preserved specimens from museum collections and 452 live specimens from High Ebro, were analysed (List S1). Preserved specimens came from the following institutions: Sociedad de Ciencias Aranzadi (Donostia), Instituto Pirenaico de Ecología, CSIC (Jaca), Departament de Biologia Animal, Barcelona University (Barcelona), Estación Biológica de Doñana, CSIC (Seville), Museu de Ciències Naturals of Barcelona (Barcelona), Monestir de Montserrat (Barcelona), Laboratoire de Biogéographie et Écologie des Vertébrés, Université de Montpellier II (Montpellier), Museo Nacional de Ciencias Naturales, CSIC (Madrid) and Departamento de Biología Animal, Salamanca University (Salamanca). The geographical location of the specimens was determined on the Universal Transverse Mercator Projection (UTM). UTM coordinates were gathered manually from map series (1 : 250 000 from Mapa Militar Digital de España v1.5). Specimens from High Ebro came from visual encounter surveys based on 1 × 1 km UTM squares and road sampling between March 2004 and August 2007. They were captured by hand and their geographical location (UTM coordinates, WGS84 datum) recorded with a Global Positioning System (GPS) with a location error less than 8 m.

For each specimen, nine meristic characters were recorded: number of ventral scales, excluding prefrontals and anal, following the method used by Saint-Girons (1978) (VENT); subcaudal scales (SUBCA); apical scales (APICA); supralabial scales (SUPRAR); infralabial scales (INFRAR); periocular scales (PERIR); loreal scales (LORER); intercantal plus intersupraocular scales (INTER); and dorsal marks (DMARK). When bilateral characters were considered on the right hand side of the head. These characters were selected because they

were reported to present variation across geographical ranges of the two vipers in previous morphological analyses (Zuffi 2002; Brito et al. 2006).

As European vipers were reported to be dimorphic (Saint-Girons 1978), morphological traits were tested for sexual dimorphism. At regional and local scales VENT and SUBCA presented significant sexual dimorphism in number (Kruskal–Wallis test,  $p < 0.001$ ), thus they were analysed separately for females (VENTF, SUBCAF) and males (VENTM, SUBCAM). In an initial stage, specimens were classified into putative species in order to identify individuals with intermediate traits. Criteria for classification included the combination of the following morphological characters (for details see Martínez-Freiria et al. 2006): (1) snout elevation and number of apical scales: snout slightly upwards with two to three apical scales in *V. aspis* and snout upwards, forming an appendix with three to nine apical scales in *V. latastei*; (2) shape of the dorsal stripe: alternated cross bands with a thin vertebral line (type 0) or narrow angular zigzag (type 1) in *V. aspis*, and wide zigzag (type 2) or rounded-rhomboidal marks running together to form a wavy or zigzag stripe (type 3) in *V. latastei*; (3) number of dorsal marks: 45–78 in *V. aspis* and 33–57 in *V. latastei*. Preliminary analyses of microsatellite variation corroborated this morphological classification between true and intermediate forms, i.e. specimens exhibiting intermediate morphological traits between both species corresponded mostly to hybrids (authors, unpublished data). Thus, intermediate vipers, 98 specimens at the regional scale and 92 at the local scale, were removed from the following analyses but their geographical location was displayed in the resulting maps.

### Modelling procedures

For the regional scale, NE Iberia, 630 vipers, 369 *V. aspis* and 261 *V. latastei*, were analysed. The locations of specimens were displayed in the GIS ArcMap 9.2 (ESRI 2006) on the WGS84 datum (Fig. 1b). Modelling analyses were based on 10 × 10 km UTM cells using the coordinates for the centroids of each grid cell ( $X$  and  $Y$  central coordinates). A total of 135 10 × 10 km UTM cells, 99 for *V. aspis* and 41 for *V. latastei*, were used in the regional scale analyses, with five grid cells presenting sympatry between both vipers. For the local scale, High Ebro, 362 vipers, 183 *V. aspis* and 179 *V. latastei*, were analysed. Specimens and their locations were inscribed in a georeferenced database and also were displayed in the GIS ArcMap 9.2 on the WGS84 datum (Fig. 1c). Modelling analyses were based on GPS locations, thus each specimen had a unique location.

Although all specimens were classified into putative species in order to detect and remove intermediate vipers, the following analyses were performed independently of species classification. For both NE Iberia and High Ebro and for each morphological trait, a continuous surface was created with ArcMap 9.2, using the Geostatistical Analyst extension (Johnston et al. 2001). A Kriging function was used as an interpolation method, using weights from a semivariogram estimated through the spatial structure of the data (Oliver 1990). The Kriging function creates continuous surfaces of morphological variability by interpolating the values of each trait from the sampled locations (Kidd and Ritchie 2000; Brito et al. in press). This procedure is useful because not all morphological traits were available for all grid cells sampled (Fig. 1), and many specimens were found dead on the road, partially damaged. For the five 10 × 10 UTM cells at the regional scale where sympatry between putative species was found, the coordinates of the centroids were dislocated 10 m for each of the specimens to avoid their spatial overlap. A number of statistical measures can be used to assess the performance of the Kriging interpolation (Tables S2 and S3). For a model that provides accurate predictions, the mean error should be close to 0, the root-mean-square error and average standard error should be as small as possible, and the root-mean-square standardized error should be close to 1 (for details see Johnston et al. 2001). Each trait map was reclassified into equal intervals between their respective maximum and minimum values for the morphological character. The reclassified surfaces were overlaid with a Boolean mask to remove areas without sampled locations.

In order to identify areas with multivariate clines and high morphological variability, continuous morphological trait surfaces derived in the previous step were exported to Idrisi Kilimanjaro 14.01

Code	Variable	Range and units for NE Iberia	Range and units for High Ebro
ALT	Altitude	0–3252 m a.s.l.	621–1150 m a.s.l.
PREC	Annual average precipitation	438–1759 mm year <sup>-1</sup>	697–829 mm year <sup>-1</sup>
PRECS	Precipitation seasonality	11–35 mm	20–23 mm
TEMP	Annual average temperature	19.9–30.0°C	22.2–24.5°C
TEMPS	Temperature seasonality	4.5–6.5°C	4.8–5.3°C
MINT	Minimum temperature of the coldest month	–12.3 to 6.8°C	–1.3 to 1.7°C
MAXT	Maximum temperature of the warmest month	9.2–30.7°C	22.7–24.5°C

Table 1. Environmental factors tested for correlation with geographical variation in morphological traits of *Vipera aspis* and *Vipera latastei* in NE Iberia and High Ebro

(Clark Labs 2003) and a spatial principal components analysis (SPCA) was undertaken. The greatest advantage of PCA is that it finds structural relationships among specimens without *a priori* subdivision into discrete groups (Thorpe 1984). The SPCA maps were created using the PCA function from Idrisi Kilimanjaro 14.01, which produced a new set of uncorrelated images, principal components (PC) maps, which explain progressively the reduced variance found in the original set of surface trait maps. PC maps were derived using only traits with meaningful spatial variation as determined by an exploratory analysis of the individual trait surfaces (Brito et al. in press). Thus, homogeneous surface traits, i.e. without spatial variation, were discarded. Trait surfaces were unstandardized and used variance/covariance matrixes, implying that the surface trait maps with a greater morphological variance had greater loading scores in the SPCA (Kidd and Ritchie 2000; Brito et al. in press). For each PC map, the loading scores were used as a measure of the association between surface trait maps and the total variance accounting for each eigenvalue was used to evaluate the level of explanation of the analysis. The Slope function was then applied to the PC maps in Idrisi GIS in order to highlight areas of great changes (clines) in the PC surfaces, i.e. the regions of sharp transition in morphological variability. Finally, PC and slope maps were exported to ArcMap 9.2 for enhanced display.

Surface trait and PC maps were tested for correlation at both scales with a set of seven environmental factors including topographical and climatic factors (Table 1). These environmental variables are known to affect the distribution of *V. aspis* and *V. latastei*, and could be related to geographical variation in morphology (Brito and Crespo 2002; Santos et al. 2006; Martínez-Freiria et al. 2008). Environmental variables with a spatial resolution of approximately 1 km<sup>2</sup> were downloaded in digital format from Worldclim v1.4 (Hijmans et al. 2005). Correlations were estimated using the Band Collection Statistics tool of ArcToolbox (ESRI 2006).

## Results

### Regional scale – NE Iberia

The continuous surfaces obtained for INFRAR, LORER, PERIR, SUBCAF, SUBCAM and SUPRAR did not display spatial variation, thus they were removed from subsequent analysis. Marked clines with a general north–south orientation were found for APICA, DMARK, INTER, VENTF and VENTM (Fig. 2). The variance accounted for the two most explicative dimensions, PC1 and PC2, on the SPCA were 84.66% and 8.62% respectively. All traits explained a significant amount of variation on the PC1 axis (Table 2) and a north–south pattern of variation was observed in PC1 (Fig. 3): low values in northern areas (north and north-east of Sierra de la Peña and Sobrarbe), high values in southern areas (south of Iberian System and Moianés) and intermediate values with gradual clines along the contact zones. Three steep continuous clines and several discontinuous clines of morphological variation were identified (Fig. 3), mainly running from west to east and dividing the study area into zones of gradual transition in morphological variability. The overlay of localities of specimens on the PC1 and slope maps allows the visualization of patterns of convergence in morphological

variability but at different levels: western (High Ebro, Iberian System) and eastern (Moianés) contact zones are more related to *V. latastei* morphological variability, whereas central contact zones (Sierra de la Peña) are more related to *V. aspis* variability (Fig. 3).

Only three significant correlations between morphological and environmental variation were found (Table 3): positive between the VENTM and PC1 with PREC, and negative between VENTM and PRECS. Nevertheless, there was also a trend for a positive relationship between DMARK and VENTF with PREC and between INTER and TEMPS, and a trend for a negative relationship between APICA and PREC and between VENTM and MAXT (Table 3).

### Local scale – High Ebro

The surfaces obtained for SUPRAR, INFRAR, PERIR, LORER, SUBCAM and SUBCAF did not display spatial variation and were removed from further analysis. Marked clines with a north–south orientation were found for APICA, DMARK, INTER, VENTF and VENTM (Fig. 4). The variance accounted for PC1 and PC2 were 89.61% and 5.36%, respectively, and all traits explained a significant amount of variation in PC1 (Table 2). A clear north–south pattern of gradual variation was observed in PC1 (Fig. 5): low values in northern areas (near to Ebro River and La Lora plateau), high values in southern areas (Masa plateau) and intermediate values with steep clines at the confluence of the Rudrón and Sedanillo rivers. Three steep continuous clines of morphological variation were displayed (Fig. 5): a northern cline delimiting an area where most *V. aspis* specimens were found, a southern cline separating an area where only *V. latastei* specimens were found, and a middle cline in the confluence of the Rudrón and Sedanillo rivers, where intermediate vipers were mostly located.

Significant positive correlations were found between DMARK and VENTM with PREC and between APICA and PC1 with TEMPS (Table 3). Significant negative correlations were found between PC1 and PREC and between DMARK with TEMP and TEMPS respectively. There was also a trend for a positive relationship between APICA and PC1 with TEMP and a trend for a negative relationship between APICA and PREC and between VENTM and TEMPS.

## Discussion

Morphological traits of *V. aspis* and *V. latastei* have been studied in discrete populations (e.g. Zuffi 2002; Brito et al. 2006; Golay et al. 2008) or using continuous surfaces derived from geostatistics (only *V. latastei*: Brito et al. in press). Nevertheless, nothing was known about morphological

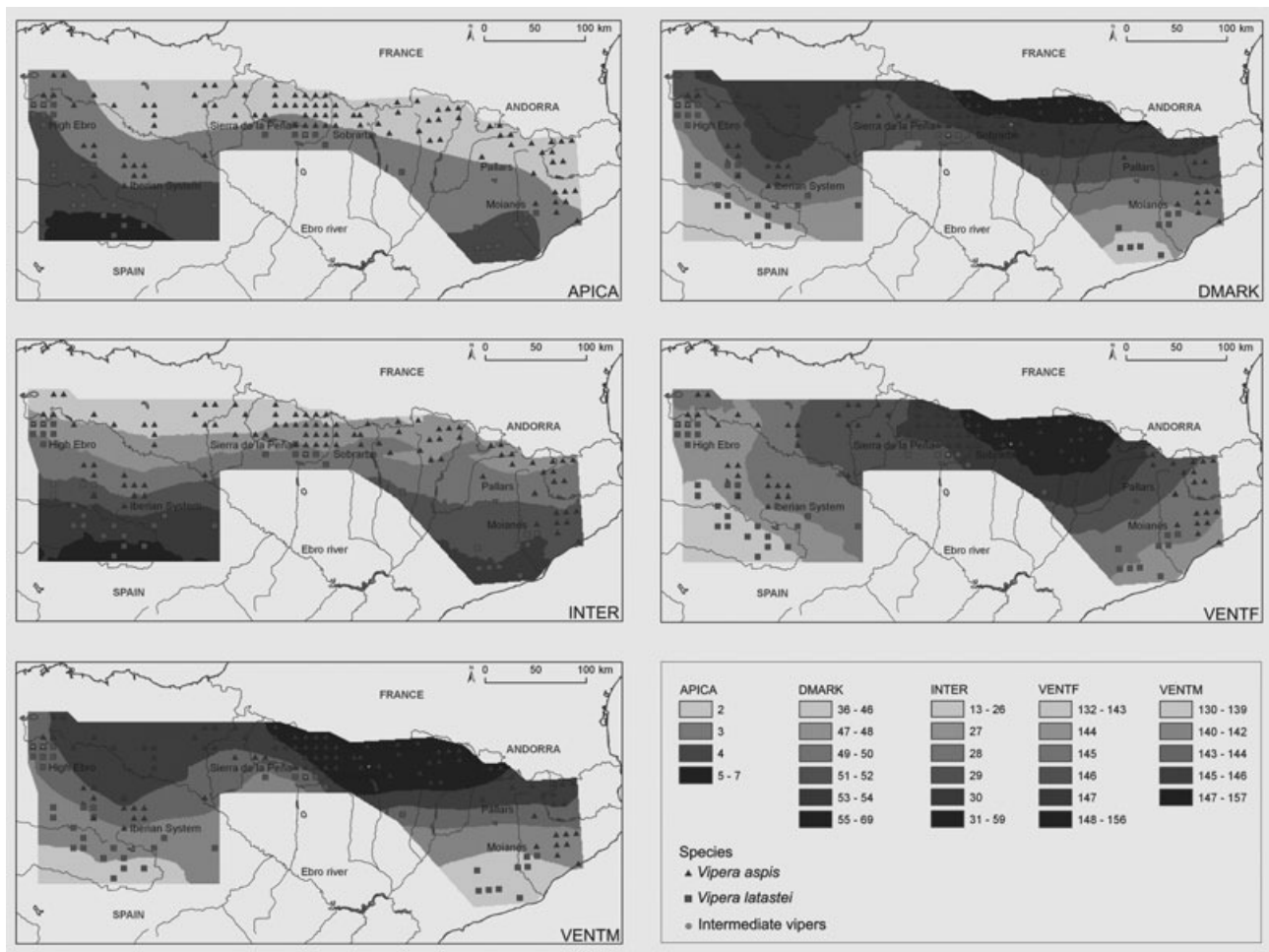


Fig. 2. Spatial variation patterns of morphological traits from *Vipera aspis* and *Vipera latastei* in NE Iberia. Continuous surfaces were derived using Kriging interpolation algorithm (see the Materials and Methods section for details). APICA, number of total apical scales; DMARK, number of dorsal marks; INTER, number of intercantal plus intersupraocular scales; VENTF, number of ventral scales for females; VENTM, number of ventral scales for males. Each symbol in the map represents a UTM 10 × 10 km cell for which specimens with morphological data were available

Table 2. Loading scores on the first two principal components extracted according to the spatial principal components (PC) analysis of individual surfaces of variation of morphological traits from *Vipera aspis* and *Vipera latastei* in NE Iberia and High Ebro and percentage of variance explained by the first two PCs

TRAIT	Regional scale: NE Iberia		Local scale: High Ebro	
	PC1	PC2	PC1	PC2
APICA	-0.927	0.084	-0.973	-0.064
DMARK	0.966	-0.036	0.971	0.064
INTER	-0.947	0.337	-0.919	-0.061
VENTF	0.796	0.570	0.904	0.390
VENTM	0.934	0.106	0.926	-0.364
% variance	84.660	8.620	89.610	5.360

variation in their contact zones and the relationship between geographical variation and environmental pressures. The combination of GIS and geostatistics proved to be a powerful tool capable of analysing morphological transition zones, identifying morphological variation patterns and establishing links between morphology and environmental factors. Furthermore, in our case study, results from local and regional

scales are mostly concordant, enabling us to draw scale-independent inferences. For instance, the major variation patterns found in this study follow the same geographical trends for *V. latastei* in the remaining distribution area (Brito et al. in press). Although continuous surfaces of morphological variation covering the entire range of *V. aspis* are not available, northern populations (France) belonging to the subspecies *V. a. aspis* (Zuffi 2002; Trutnau et al. 2005) also follow the same geographical trends reported in the present study. These techniques could be used to analyse other contact zones between European viper species, such as between *V. berus* and *V. aspis* in the Atlantic Loire of France (Saint-Girons 1975), between *V. ammodytes* subspecies in the Balkans (Tomović 2006), or even between complex groups such as Iberian *Podarcis* lizards (Pinho et al. 2007).

**Morphological variation**

Artefacts in surface trait maps are relatively common (Kidd and Ritchie 2000; Brito et al. in press) and a few concave and fanning out cross-hatching artefacts can be observed in areas without sampled localities in the surface for INTER in NE Iberia and in several trait surfaces in High Ebro. Sampled

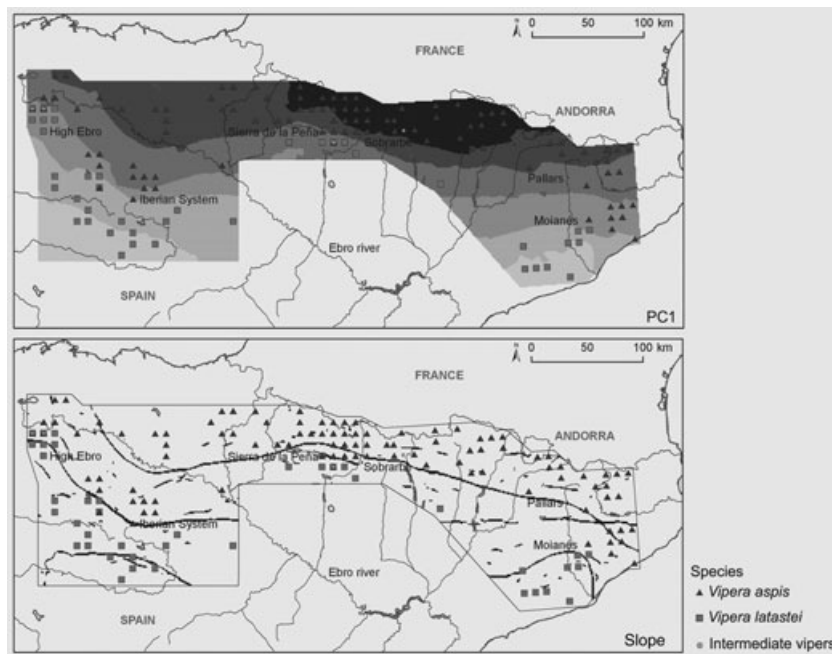


Fig. 3. Spatial principal component map (PC1) and clines of steep transition (SLOPE) for morphological variability of *Vipera aspis* and *Vipera latastei* in NE Iberia. Each symbol in the map represents a UTM 10 × 10 km cell for which specimens with morphological data were available

Table 3. Correlations between environmental factors and morphological trait surfaces and morphological variability (PC1) of *Vipera aspis* and *Vipera latastei* in NE Iberia and High Ebro.

Scale	Trait	ALT	PREC	PRECS	TEMP	TEMPS	MINT	MAXT
Regional: NE Iberia	APICA	-0.176	-0.722	0.486	0.572	0.555	0.151	0.516
	DMARK	0.358	0.734	-0.698	-0.441	-0.528	-0.386	-0.629
	INTER	-0.136	-0.655	0.493	0.535	0.718	0.144	0.484
	VENTF	0.363	0.708	-0.595	-0.336	-0.225	-0.437	-0.602
	VENTM	0.501	0.757*	-0.766*	-0.368	-0.459	-0.544	-0.716
Local: High Ebro	PC1	0.329	0.789*	-0.667	-0.509	-0.568	-0.354	-0.647
	APICA	0.195	-0.734	-0.172	0.709	0.762*	-0.307	0.487
	DMARK	-0.219	0.762*	0.187	-0.753*	-0.803*	0.337	-0.499
	INTER	0.097	-0.676	-0.096	0.573	0.631	-0.196	0.483
	VENTF	-0.145	0.605	0.125	-0.552	-0.649	0.227	-0.400
	VENTM	-0.111	0.795*	0.091	-0.689	-0.700	0.239	-0.576
PC1	-0.173	0.775*	0.150	-0.720	-0.770*	0.291	-0.529	

\*Correlations above 0.750 identify environmental factors related to morphological variation (See Materials and Methods and Table 1 for description of the variables).

localities in NE Iberia are roughly distributed evenly whereas in High Ebro, morphological data are concentrated along the river valleys and mostly absent from plateaus (Fig. 1). This is a consequence of the distribution of vipers at a local scale, which tend to be absent from the windy and well-exposed plateaus (Martínez-Freiría et al. 2006, 2008). Despite the presence of artefacts in some individual surface trait maps, general patterns of morphological variation can be identified and tested for correlation with environmental factors.

In both NE Iberia and High Ebro, there is a north–south gradual gradient in morphological variation. There are marked morphological differences between the populations of *V. aspis* in the north and of *V. latastei* in the south, but both species exhibit morphological convergence in the contact areas, precisely where intermediate vipers are found. At the regional scale, there is a sharp transition in morphological variability, but areas of integration and convergence are identified in almost all contact zones, such as the case of the High Ebro, where *V. aspis*, *V. latastei* and intermediate vipers are present. These patterns of morphological variation are typical of

secondary contact zones between allopatric forms, where a rapid transition area with steep clines of morphological variation between relatively stable geographical forms can be recognized (Thorpe 1987).

#### Correlates between morphological variation and environmental factors

The current study identified significant correlations, mostly in High Ebro, between morphological variability of some traits and environmental factors. This finding suggests that some morphological traits are under selection and may display adaptations to local environmental conditions. For instance, the number of dorsal marks is positively related to precipitation and negatively related to temperature and temperature seasonality at the local scale, exhibiting larger numbers in northern areas. Accordingly, geographical variation in the number of dorsal marks of *V. latastei* parts of Iberia other than high Ebro and North Africa follows the trend of decreasing number of marks with latitude (Brito et al. in

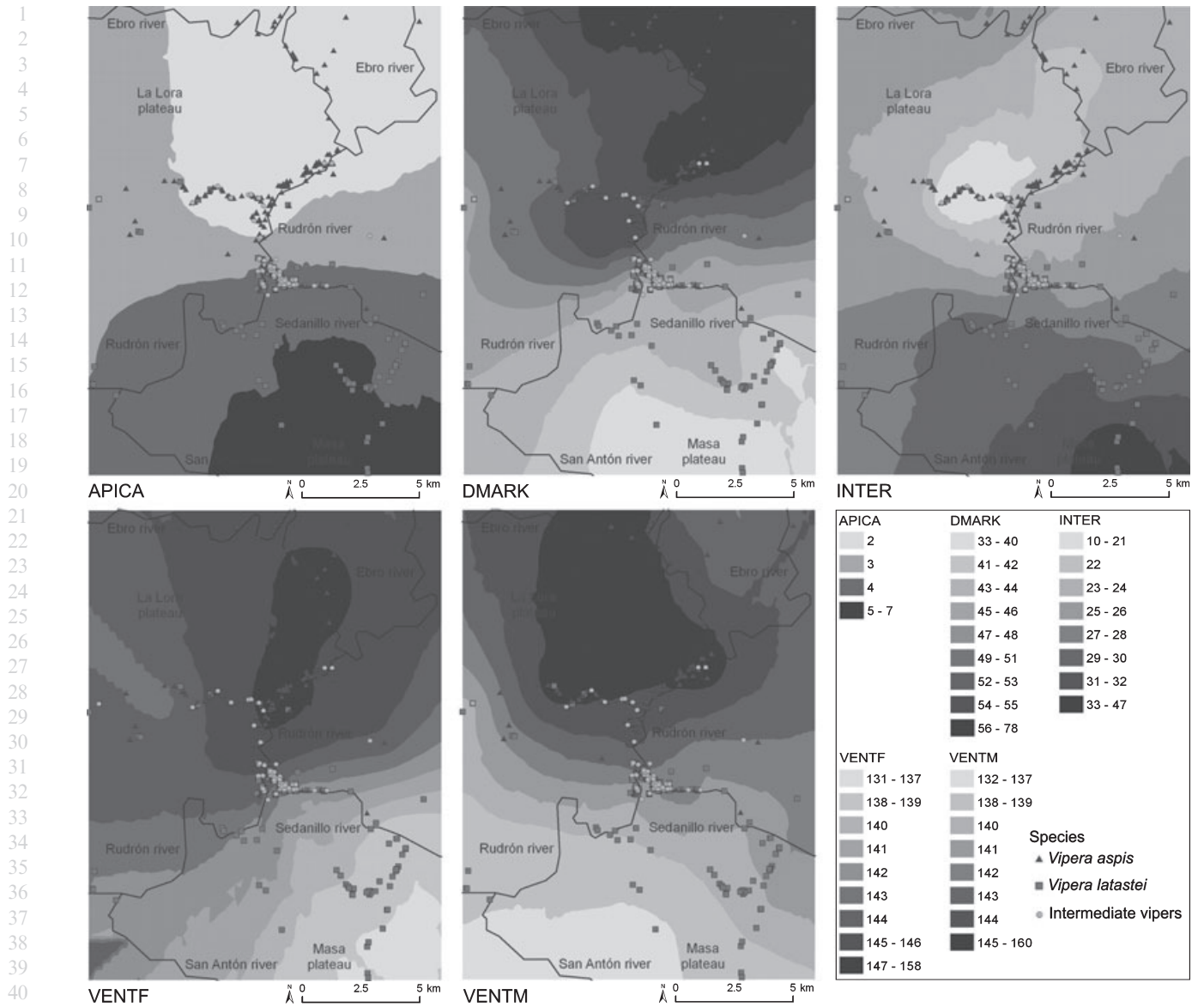


Fig. 4. Spatial variation patterns of morphological traits from *Vipera aspis* and *Vipera latastei* in the High Ebro. Continuous surfaces were derived using Kriging interpolation algorithm (see the Materials and Methods section for details). APICA, number of total apical scales; DMARK, number of dorsal marks; INTER, number of intercantal plus intersupraocular scales; VENTF, number of ventral scales for females; VENTM, number of ventral scales for males. Each symbol in the map represents a GPS location for which specimens with morphological data were available

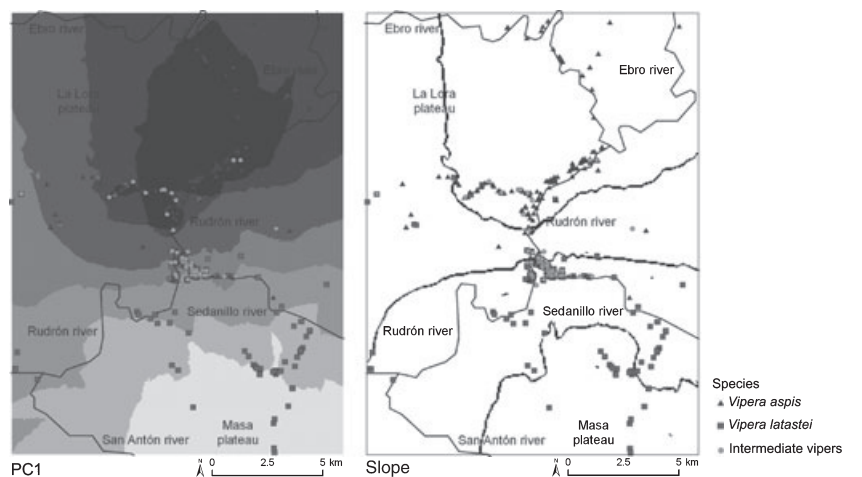


Fig. 5. Spatial principal component map (PC1) and clines of steep transition (SLOPE) for morphological variability of *Vipera aspis* and *Vipera latastei* in the High Ebro. Each symbol in the map represents a GPS location for which specimens with morphological data were available

press). Variations in the dorsal pattern of European vipers could reflect mimicry or cryptic adaptation of snakes to the substrate (Saint-Girons 1980; Nikasnen and Mappes 2005), because vipers are terrestrial snakes with sedentary behaviour and individuals tend to remain inside bushes through the year (e.g. Brito 2003a, b). Therefore, vipers with a higher number of dorsal marks may camouflage more efficiently in habitats with denser vegetation, in comparison with vipers with a lower number of dorsal marks, which camouflage better in areas of sparse vegetation. Precipitation is one of the main factors that influences vegetation (Costa et al. 1996) and could act as a surrogate for the amount of vegetation covering the substrate. Northern localities, with Atlantic climate and high levels of precipitation, usually present denser vegetation than southern localities, with Mediterranean climate and reduced precipitation (Fig. S4). In fact, populations from northern areas belonging to *V. aspis*, especially at the local scale, presented higher number of dorsal marks, which should be related to higher precipitation levels resulting in dense vegetation cover. Intermediate vipers are found in areas of climate transition, i.e. in areas with intermediate levels of precipitation and probably vegetation cover (Fig. S4).

The number of ventral scales is positively related to precipitation at both scales and negatively related to precipitation seasonality at the regional scale, exhibiting larger numbers in northern areas. Furthermore, there is a trend for a negative relationship between number of ventral scales and maximum temperature at regional scale and temperature seasonality at the local scale, the later being higher in southern areas (Fig. S4). As with the number of dorsal marks, the variation in the number of ventral scales of *V. latastei* in parts of Iberia other than high Ebro and North Africa follows the same pattern (BrITO et al. in press). The number of ventral scales is related to the number of vertebrae in European vipers (Saint-Girons 1978, 1980). Both vertebrae and ventral scales are linked by connective tissue and muscles and they form an interactive unit that is responsible for locomotion (Cundall 1987). Variation in the number of vertebrae-ventral scales could be related to climatic conditions during embryonic development because they can exert profound and long-term effects on many types of organisms. Although viviparity in ectotherms allows the reproducing female to buffer ambient thermal variation for the developing offspring, even an actively thermoregulating female may be unable to provide optimal incubation regimes in severe weather conditions (Lourdais et al. 2004). Many ectotherms have a negative relationship between developmental temperature and vertebral number (e.g. in fishes, Fahy 1972; or in salamanders, Peabody and Brodie 1975), but snakes have been reported to follow the opposite trend (Fox 1948; Lindell 1994). In fact, warmer temperatures during gestation were demonstrated to increase ventral scale counts in *V. aspis* neonates (Lourdais et al. 2004). Other selective pressures, such as water exchange processes across the skin (Sanders et al. 2004) or locomotor performance through a given substrate (Kelley et al. 1997), were reported to affect the number of ventral scales in snakes: higher numbers in drier and open habitats. However, *V. aspis* and *V. latastei* follow again an opposite trend, exhibiting lower number of ventrals in drier and open habitats. Thus, phylogenetical relationships should also be related to the complex pattern of variation observed.

At both scales, geographical variation in morphological variability of vipers and their morphological convergence in contact zones seem to be an adaptation to environmental

selective pressures, such as precipitation and temperature. Nevertheless, correlations with other environmental factors are generally weak ( $r < 0.700$  in most cases) and several morphological traits with spatial meaning, such as the number of intercantals plus intersupraoculars and apicals, are not related to the examined environmental factors. Thus, other selective environmental pressures not discussed in our study, biotic factors, reinforcement or even gene flow processes, may be also playing an important role in the geographical variation of both species. However, low growth rates, delayed sexual maturation and low reproductive frequency of Iberian vipers combined with general rareness and conservation status (Pleguezuelos et al. 2002; Santos et al. 2006) hamper the development of experimental studies enlightening biological responses of different phenotypes to environmental pressures. Considering the common evolutionary history of these species, the importance of gene flow and hybridization can be perceived. *Vipera aspis* and *V. latastei* are Mediterranean species that evolved from a common ancestor in Western Europe in early Miocene and probably suffered allopatric speciation during a probable allopatric speciation process during the Pleistocene ice ages of the Quaternary (Lenk et al. 2001; Szyndlar and Rage 2002; Garrigues et al. 2005). Consequently, their current distributions are mainly parapatric and sympatry is almost non-existent (Saint-Girons 1980). Thus, hybridization could be expected when populations from both species meet in secondary contact. In fact, gene flow occurs in the sympatry area of High Ebro (authors, unpublished data). Also, the analysis of geographical variation of 10 morphological traits of *V. latastei* with geostatistics in the Iberian Peninsula and North Africa demonstrated that populations from NE Iberia have a high level of morphological differentiation and could be the result of introgression with *V. aspis* (BrITO et al. in press). Thus, where environmental factors allow contact between *V. aspis* and *V. latastei*, hybridization and interspecific gene flow occur and interact with phenotypic variation due to environmental pressures.

## Conclusions and future research

In summary, the current study identified a convergent north-south pattern in morphological variability in *V. aspis* and *V. latastei* in their contact area in the Iberian Peninsula. Correlations between geographical patterns in morphological traits with abiotic environmental selective pressures were found, suggesting that these patterns could result from local environmental selective pressures. Nevertheless, environmental factors seem to act over particular traits whereas other factors such as biotic pressures or gene flow could be also responsible for morphological variability and convergence between these species. Comparative studies on the ecology of both species, allowing the testing of interspecific competition, and genetic studies, allowing the quantification of interbreeding and gene flow, are needed to draw evolutionary scenarios for these vipers.

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## Resumen

Patrones geográficos de variación morfológica y correlaciones ambientales en zonas de contacto: una aproximación multi-escalar usando dos víboras mediterráneas

La adaptación a presiones selectivas similares puede explicar la convergencia morfológica entre especies cercanas en las zonas de contacto. Se han usado la Geostatística y los SIG tanto para identificar a patrones multi-escalares en la variabilidad morfológica como para testar la hipótesis de una convergencia morfológica debido a presiones ambientales locales en las zonas de contacto entre *Vipera aspis* y *Vipera latastei* en la Península Ibérica. Nueve rasgos morfológicos de 630 y 362 víboras a escala regional y local, respectivamente, han sido interpolados por Kriging para generar superficies de variación morfológica. Kriging es un algoritmo geostatístico que permite investigar la estructura de los datos a través de modelos estadísticos. A ambas escalas se han observado patrones convergentes con dirección norte-sur en la variabilidad morfológica y las zonas de contacto han sido identificadas como zonas de integración donde se encuentran víboras con caracteres morfológicos intermedios. Se han hallado correlaciones significativas entre las superficies univariadas y multivariadas con la precipitación y la temperatura estacional. Por lo tanto, varios rasgos morfológicos están aparentemente bajo selección ambiental local. Sin embargo, la influencia de presiones bióticas y flujo genético en la convergencia morfológica de las víboras en las zonas de contacto necesita estudios adicionales.

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## Supporting Information

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

List S1. Catalogue number of examined specimens during the fieldwork in High Ebro and in herpetological collections, divided by species, source (bold) and sex.

Table S2. Statistical measures to assess the performance of the Kriging interpolation of morphological traits from specimens of *Vipera aspis* and *Vipera latastei* in NE Iberia. N UTM cells refer to the number of 10 × 10 km UTM cells for which data were available for a given trait

1 Table S3. Statistical measures to assess the performance of  
2 the Kriging interpolation of morphological traits from spec-  
3 imens of *Vipera aspis* and *Vipera latastei* in High Ebro. N GPS  
4 locals refer to the number GPS localities for which data were  
5 available for a given trait

6 Figure S4. Average annual precipitation (PREC) and  
7 temperature seasonality (TEMPS) of the study areas. These  
8 environmental variables were mostly correlated with geo-  
9 graphical variation in morphological traits of *Vipera aspis* and

*Vipera latastei* in NE Iberia and High Ebro. Each symbol in  
the map represents a 10 × 10 km UTM cell (NE Iberia) or a  
GPS location (High Ebro) for which specimens with morpho-  
logical data were available

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Change bold to non-bold type	(As above)	⊖
Insert 'superior' character	/ through character or ∧ where required	Υ or Υ under character e.g. Υ or Υ
Insert 'inferior' character	(As above)	∧ over character e.g. ∧
Insert full stop	(As above)	⊙
Insert comma	(As above)	,
Insert single quotation marks	(As above)	Ƴ or ƴ and/or ƶ or Ʒ
Insert double quotation marks	(As above)	ƶ or Ʒ and/or Ʒ or ƶ
Insert hyphen	(As above)	⊥
Start new paragraph	┌	┌
No new paragraph	┐	┐
Transpose	└┘	└┘
Close up	linking ○ characters	Ⓞ
Insert or substitute space between characters or words	/ through character or ∧ where required	Υ
Reduce space between characters or words		↑