The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2014, **112**, 180–203. With 7 figures

Ecological preference between generalist and specialist rodents: spatial and environmental correlates of phenotypic variation

JUAN J. MARTÍNEZ[1,2*], VIRGINIE MILLIEN[2], IVANA SIMONE[1] and JOSÉ W. PRIOTTO[1]

[1]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Departamento de Ciencias Naturales, Universidad Nacional de Río Cuarto, Agencia Postal N°3, 5800, Río Cuarto, Córdoba, Argentina*
[2]*Redpath Museum, McGill University, 859 Sherbrooke Street West, Montreal, Québec H3A 0C4, Canada*

Received 26 September 2013; revised 9 January 2014; accepted for publication 9 January 2014

Different ecological preferences among species may result in differences in response to similar environmental variation. To test this hypothesis, we assessed the patterns of skull and mandible size and shape variation in three Sigmodontinae mice from agroecosystems of central Argentina with increasing degree of specialization: *Calomys musculinus*, *Akodon azarae* and *Oxymycterus rufus*. Spatial patterns in size and shape were analysed after controlling for allometry and sexual dimorphism using a total of 697 specimens. We then evaluated the covariation between shape, climatic and environmental variables and assessed the contribution of distinct climatic and environmental variables to phenotypic variability. *Oxymycterus rufus* displayed a marked spatial structure, and there was a high correlation between shape, climatic and environmental variables in this species. Climatic and environmental variables had a moderate effect on the phenotype of *A. azarae*, and were not correlated with morphological variation in *C. musculinus*. Our study highlights the difference in phenotypic responses to spatial and environmental gradients across coexisting species, specialist species displaying a more marked spatial structure in morphology than generalist species.

ADDITIONAL KEYWORDS: agroecosystems – geometric morphometrics – local variation – mandible – skull.

INTRODUCTION

Specialist species are considered to possess a reduced or limited ecological niche breadth. As proposed by Grinnell (1917), the ecological niche of a species can be defined by a given set of variables or resources of its habitat. Habitat-specialization can be a proxy for the Grinnellian niche if habitat is not only the physical place where the species is found but also encompasses other abiotic and biotic conditions that influence species' persistence (Devictor *et al*., 2010).

The alteration and fragmentation of a habitat is expected to be reflected in the habitat specialization of individuals facing such changes in their environment. One potential outcome of habitat fragmentation is the development of spatial structure in populations, with the organization of individuals with similar morphological and genetic characteristics into defined spatial units (Dujardin, 2008; Ledevin & Millien, 2013; Rogic *et al*., 2013). Studies of the spatial structure at the landscape scale can increase our understanding of the mechanisms of persistence and habitat specialization of natural populations in agricultural landscapes.

In contrast to geographical patterns of morphometric variation at regional scales (e.g. Renaud & Millien, 2001; Polly, 2003; Renaud & Michaux, 2003; Caumul & Polly, 2005; Macholán, Mikula & Vohralík,

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*Corresponding author. E-mail: juan_jmart@yahoo.com.ar

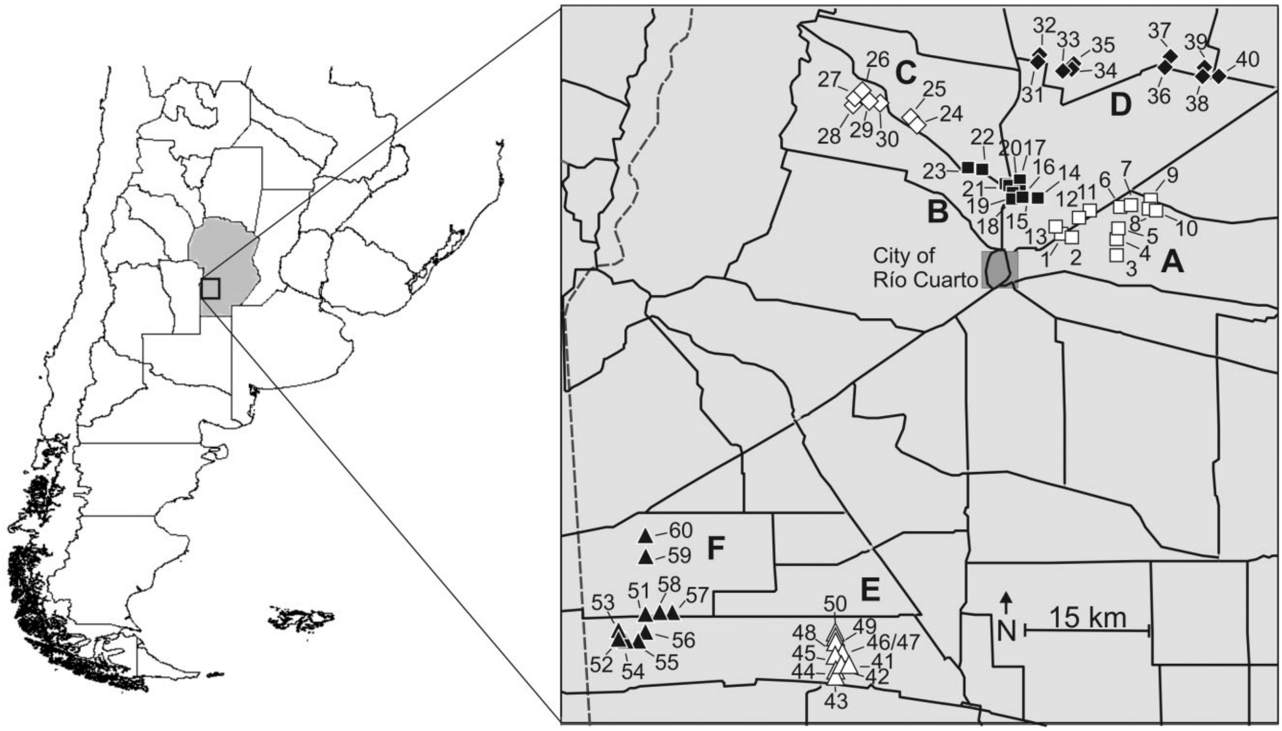

Figure 1. Geographical position of sampling sites located in central Argentina (Córdoba province).

2008; Colangelo *et al*., 2010; Piras *et al*., 2010; Martínez & Di Cola, 2011), there are fewer studies on such variation at local or fine spatial scales among rodent populations. Tolliver *et al*. (1987) used linear measurements of the skull of *Peromyscus leucopus* and did not find any morphological differences between populations in a small geographical area (< 12 km^2) in Kansas. At a larger scale, Le Boulengé *et al*. (1996) showed that, despite the absence of noticeable environmental heterogeneity within an area of 150 km^2 in Belgium, the studied populations of *Ondatra zibethicus* presented significant differences among them. More recently, Lalis *et al*. (2009) found both genetic and morphometric differences between two adjacent populations of the African *Mastomys natalensis* separated only by 5 km. Similarly, Ledevin & Millien (2013) and Rogic *et al*. (2013) detected, both at the morphological and at the genetic levels, a strong pattern of geographical differentiation among populations of the white-footed mouse (*P. leucopus*) within an agricultural landscape covering an area of approximately 630 km^2 in southern Quebec.

Agroecosystems are an ideal system to study the effects of the spatial variability in the environment on population phenotypic structure, particularly for small mammals with different levels of habitat specialization. In recent decades, the rate of agricultural expansion has increased considerably in Argentina due to technological changes and market conditions. The intensification of agriculture has fragmented the natural habitat and land-use changes in the Pampean region (Baldi & Paruelo, 2008). Such an increase in agriculture practices seems to have reduced small mammal diversity and abundance in the Pampean region (Soriano, 1991; Hall *et al*., 1992; Medan *et al*., 2011), favouring more habitat generalist species such as the sigmodontine rodent species *Calomys musculinus* and *C. laucha*, at the expense of habitat specialist species such as *Akodon azarae* (Bilenca & Kravetz, 1995; Cavia *et al*., 2005; Fraschina, León & Busch, 2012).

The sigmodontines belong to a Neotropical subfamily of mice with about 400 living species that occupy a wide range of habitats. The small mammal assemblage covered in our study area (Fig. 1) is represented mainly by *Calomys musculinus*, *C. venustus*, *C. laucha*, *Akodon azarae*, *A. dolores*, *Oxymycterus rufus* and *Oligoryzomys flavescens* (Polop & Sabattini, 1993; Simone *et al*., 2010), as well as small didelphid marsupials such as *Thylamys pallidior* and *Monodelphis dimidiata*. *Akodon azarae* and *C. musculinus* are the most abundant species in the assemblage (Simone *et al*., 2010). *Akodon azarae* is dominant in more stable habitats such as native grasslands, railway roads, fence lines and border lines (Polop, 1996; Suárez & Bonaventura, 2001; Gomez *et al*., 2011). *Calomys musculinus* not only uses similar habitats to *A. azarae* but also thrives in highly modified habitats. Thus, this species is considered a habitat generalist (Busch & Kravetz, 1992; Mills *et al*., 1992; Busch *et al*., 1997, 2000; Ellis *et al*., 1998). Ecological changes due to the agricultural development of the Pampean region may have favoured *C. musculinus* because this species is captured in higher proportion in agroecosystem assemblages than in the undisturbed native grassland assemblage (Bilenca & Kravetz, 1995). This is because *C. musculinus* has a wider habitat and trophic niche than other coexisting rodent species in agrarian systems of central Argentina (Busch *et al*., 2000). *Oxymycterus rufus* is the largest sigmodontine in the agroecosystem of central Argentina and is a semi-fossorial, specialized insectivorous species exclusively captured in remaining natural habitats in agroecosystems (Barlow, 1969; Kravetz, 1972; Dalby, 1975; Bilenca *et al*., 2007). This rodent is thus considered a habitat specialist.

Our objective was to assess the effect of spatial and environmental factors on the morphological variation at the local scale in three species of sigmodontine rodents from the agroecosystems of central Argentina. We used geometric morphometric analyses to quantify the morphological variation of the skull and the mandible. We first evaluated the spatial patterns of size and shape variation, then estimated the relationships between shape variation, and climatic and environmental variables.

We predict that the strength of the effect of spatial and environmental variables on the phenotype of these species is conditioned, at least in part, by their degree of specialization in habitat use. We expect specialist species, such as *O. rufus* and *A. azarae*, to show more differentiation and a stronger spatial structure than *C. musculinus*, a more generalist species.

MATERIAL AND METHODS

STUDY AREA AND SAMPLING

Small mammals were captured in southern-central Córdoba province (Argentina) (Fig. 1) in April 2009. This region belongs to the Espinal ecoregion (Burkart *et al*., 1999), and its physiognomy has undergone a marked transformation due to intensive agriculture and livestock practices. The main landscape consists of a matrix of crop fields bordered by linear habitats supporting plant communities with a mix of native species and invasive weeds that provide a more stable coverage and refuge to small mammals than the crop fields. The agroecosystem has maintained some features of the original landscape in the south-west, due to the presence of dunes with native grasslands and forest patches of *Prosopis* species.

We performed removal sampling at 60 trap-lines during three consecutive weeks in April 2009 (austral autumn) in two regions: one with high land-use intensity in the north (40 trap-lines) and another one with less land-use intensity in the south (20 trap-lines) (Fig. 1). Each week, we set 20 lines of 20 live traps separated by a distance of 10 m. Each line was set up in linear habitats (field borders) located between fields and secondary roads. The trap-lines were separated by at least 1 km and were considered as localities (sites) in our analyses. Each trap was georeferenced and the traps were active for four consecutive nights.

The collected animals were carried to the laboratory for identification, sexed by visual inspection and weighed, and two external measurements were taken: body and tail lengths. Skulls and mandibles were prepared using a colony of dermestid beetles at the laboratory.

ENVIRONMENTAL VARIABLES

Landsat Thematic Mapper satellite images corresponding to fieldwork dates were analysed using ENVI 3.5 (System Research) following Simone *et al*. (2010) to estimate temperature (land surface temperature, LST), vegetation (normalized by difference vegetation index, NDVI) and 11 indices of habitat heterogeneity (HI1–11) for each trap-line.

LST is the thermal emission of the surface. It is obtained from Landsat thermal-infrared band (TIR, band 6) and it is expressed in Kelvin degrees: $T_K = K_2/[\ln(K_1 L^{-1} + 1)]$ where $L = 0.0056322 * \mathrm{TIR} + 0.1238$ (Markham & Barker, 1986), $K_1 = 60.776$ and $K_2 = 1260.56$ (Schott & Volchok, 1985; Wukelic *et al*., 1989). The temperature values were transformed to degrees Celsius as $T_C = T_K - 273$, and represented average estimates for the period of trapping.

NDVI is a type of spectral vegetation index derived from the red/near-infrared reflectance ratio. Mean NDVI is positively related to the level of photosynthetic activity, green leaf biomass, fraction of green vegetation cover and annual net primary productivity (Tucker *et al*., 1986; Myneni *et al*., 1995); NDVI ranges from −1 to +1 with an increase in green vegetation (Tucker *et al*., 1986).

In addition, a set of 11 heterogeneity indices were defined to assess the landscape structure, all based on the quantification of the number of different classes included in an observational kernel (García-Gigorro & Saura, 2005; Baldi, Guerschman & Paruelo, 2006; Mora *et al*., 2010). To calculate these indices, one size kernel of 6 × 6 pixels (180 × 180 m or 3.24 ha) was delimited around each trap line. HI1 considered the number of different classes inside the kernel from a classification image, obtained by the Isodata method from a combination of Landsat bands 3–4–5. HI2 was calculated following Mora *et al*. (2010). HI1 is similar to HI2, but the latter relativized to the kernel area. HI3, HI4 and HI5 considered the number of different classes inside the kernel from a classification image from Landsat bands 1, 3 and 4, respectively. HI6–11 were based on the number of 'border-type' pixels included in the kernel for images that were filtered from different raw Landsat bands by using the high-pass Sobel filter. For Sobel filtering calculations, see Simone (2010). Finally, the last structural landscape heterogeneity variable considered was rainfall, obtained for each region from the values of the 3 months previous to the trapping session (rian.inta.gov.ar/agua/bdmet.aspx). Three additional local heterogeneity variables related to habitat fragmentation were used: field border width and height (BW; BH) and roadside width (RSW). BW (m) was the distance from the field wire fences to the road. BH (m) was measured from the road line up to the base of the wire fences.

To reduce the parameters included in further analyses, we conducted pairwise correlations on standardized scores of the variables (Z scores). We used Pearson's $r = 0.75$ as a cut-off to identify highly correlated variables (Riesler & Apodaca, 2007). Over the 17 original variables, we only used 13 for further analyses (four HIs were discarded due to their high correlation). The four discarded variables represented variants of those employed for further analyses. The pairwise correlations and their statistical significance are shown in Table S1.

SPECIMENS AND MORPHOMETRIC METHODS

The geometric morphometric analyses were performed on adult specimens (upper third molar and lower third molar completely erupted) of *C. musculinus* (173 males and 159 females for skull; 159 males and 140 females for mandible), *A. azarae* (161 males and 130 females for skull, 155 males and 129 females for mandible) and *O. rufus* (46 males and 28 females for skull and mandible). All the specimens, 697 in total for the three species (Table S2), are stored at the Colección de Mamíferos de la Universidad Nacional de Río Cuarto (CUNRC), Córdoba, Argentina. The specimens of *Calomys* were identified to the species level using multivariate analysis on three external and 17 cranial and mandible linear measurements, and further validated with a visual examination of external features on the specimens as well as ongoing genetic analyses (L. Sommaro *et al*., unpubl. data; M. B. Chiappero *et al*., unpubl. data).

Ventral images of the skull and labial view of right hemi mandible were taken by one of the authors (J.J.M.) using a flat bed Epson V300 Photo scanner under standardized conditions, with a resolution of 6610 × 5727 pixels. Images for all the specimens were taken twice in a random order in two different scanning sessions. Similarly, the digitization of landmarks was performed twice in a random sequence to account for the measurement error due to positioning, digitization, and matching of the skull and mandible configurations. Landmarks from the ventral view of skulls were taken only on the right side to avoid and minimize the influence of asymmetry on landmark configurations.

The landmarks were chosen for their positional homology and easiness to identify. All landmarks were of types I or II, according to Bookstein (1991). Sixteen and 11 landmarks were digitized on the ventral view of the skull and the lateral view of the mandible, respectively, for the three species (Fig. S1). In *O. rufus*, mandible morphology of which is slightly different, some landmarks were different from those digitized in the other two species. However, the landmarks used covered the whole morphology of the mandible and we were thus able to compare the variation in mandible shape across the three species.

Landmark configurations for the skulls and mandibles for the three species (two replicates for each specimen) were scaled to unit centroid size and superimposed using the least-square generalized Procrustes method (Rohlf & Slice, 1990). We performed independent analyses for each species and structure. The centroid size was estimated as the square root of the sum of squares of the distances between each landmark and the centroid of the configurations of landmarks (Bookstein, 1991). This variable was significantly correlated with body mass and body length (Table S3), and was used as a proxy for size in further analyses.

A one-way analysis of variance (ANOVA) using specimen as a factor and centroid size of both replicates as an independent variable was performed to estimate measurement error on size following Yezerinac, Lougheed & Handford (1992). Similarly, we performed a Procrustes ANOVA (Klingenberg & McIntyre, 1998) on aligned coordinates taking into account the effect of specimen for measurement error in the shape data. After accounting for measurement error in size and shape, the replicates for each specimen were averaged and the mean was used in further analyses.

To control for allometric effects (log centroid size) on shape variables we performed multivariate regressions. The null hypothesis of independence between size and shape was estimated using 10 000 permutations. When we obtained significant allometric effects, the residuals of multivariate regression were used as size-free shape variables.

Sexual dimorphism in size and shape was evaluated by ANOVA and multivariate analysis of variance

(MANOVA), respectively. The variance accounted for by sex differences was estimated by linear regressions using sex as a dummy variable. Principal component analyses (PCAs) were conducted on mean size-free shape coordinates per trap-line (locality) and on individual scores to summarize the phenotypic variation across localities and the overall variation, respectively.

Levels of intraspecific variation for the three species were investigated using coefficients of variation for the skull and mandible size. For shape we plotted on the same scale the scores of the first two principal components derived from independent analysis for each species following Dayan, Wool & Simberloff (2002). We expected specialized species to have lower levels of variability than generalist species.

Statistical analyses of phenotypic variation in space and across environments

The joint and partial contribution of spatial, climatic and environmental variables on size and shape variation for the three species was evaluated using variation partition analysis (Peres-Neto *et al*., 2006), which uses multiple and partial regression techniques to evaluate the contribution of explanatory data sets on the response variables. The method uses linear models for univariate variables (i.e. size) and canonical redundancy analysis for multivariate response variables (i.e. shape). For each dependent data set, a 'pure' component is computed and is interpreted as the variance of the response variable explained by the independent variable when its interactions with other independent variables are accounted for (Piras *et al*., 2010). The significance of full and pure contributions of explanatory variables was estimated by 10 000 permutations. In this analysis, the significance of shared fractions between explanatory data sets cannot be tested (Borcard, Gillet & Legendre, 2010).

Spatial variation

The patterns of size and shape spatial variation were analysed using the Moran eigenvector map (MEM) method (Bertin *et al*., 2012). This method allows modelling non-directional trends of spatial variation (Dray, Legendre & Peres-Neto, 2006; Blanchet, Legendre & Borcard, 2008; Blanchet *et al*., 2011). The MEM analysis is based on eigenvector decomposition of the relationships among sites (individual georeferenced traps in our study), and has two components: (1) a list of links among sites represented by a connectivity matrix and (2) a matrix of weights to be applied to these links. Dray *et al*. (2006) pointed out that MEM bears an immediate connection with Moran's I spatial autocorrelation index and can be modulated to optimize the construction of spatial variables. MEM produces $n-1$ (where n = number of sites) spatial variables with positive and negative eigenvalues, allowing the construction of variables modelling both positive and negative spatial correlation. The connectivity matrices were obtained by Delaunay triangulation of geographical coordinates of georeferenced traps for the three species for size (log centroid size) and shape (principal components scores of size-free coordinates) independently. For the site-to-site distances, we used three different schemes: a binary (i.e. two sites are either connected or not according to Delaunay triangulation) and a weighted scheme that represents the likelihood of exchange between sites connected by links. This approach assumes that the ecological similarity between two sites is higher for site pairs that are spatially closer. We used the following function $f = 1 - (d/d_{\max})^{\alpha}$ where d is a distance value and $d_{\max}$ is the maximum value in the distance matrix; $\alpha = 1$ and 2 (Dray *et al*., 2006; Blanchet *et al*., 2011; Bertin *et al*., 2012) where $\alpha = 1$ corresponds to a linear function and $\alpha = 2$ corresponds to an exponential function, assuming that ecological similarity between sites decreases as the square of the geographical distance. For each candidate scheme we computed MEM eigenfunctions, reordered them according to their explanatory power and retained the model with the lowest corrected Akaike information criterion (AICc). Only eigenvectors with positive and significant spatial autocorrelation were considered. Shape changes across the spatial variables were depicted by means of linear regression. Lastly, the overall differentiation between northern and southern localities in skull and mandible size was estimated by one-way ANOVA.

Effect of the environment

We performed two-block-partial least squares (2B-PLS) analyses (Rohlf & Corti, 2000) to determine the covariation between shape variables and 13 climatic and environmental variables, considering either the mean value per trap line or individual scores in two separate analyses. The 2B-PLS method constructs pairs of vectors or latent variables that represent linear combinations of the original variables in each data set (i.e. the environmental and climatic data set and the shape data set). These pairs have to account for as much of the covariation between the two data sets as possible. The significance of the association of morphometric and both climatic and environmental variables was obtained by 10 000 permutations. The analyses were carried out using the pooled within-group PLS analysis option for sexes in *A. azarae* and *C. musculinus*. This option is used when there are

Table 1. Biodiversity indices, number of individuals and relative abundance (%) of species captured in the regions with different land-use intensity

	Land-use intensity	
	High	Low
Akodon azarae	206 (32.49%)[a]	93 (40.43%)
Akodon dolores	4 (0.63%)	8 (3.48%)
Calomys laucha	11 (1.74%)	–
Calomys musculinus	302 (47.63%)	44 (19.13%)
Calomys venustus	56 (8.83%)	2 (0.87%)
Graomys griseoflavus	–	5 (2.17%)
Necromys lasiurus	–	24 (10.43%)
Oligoryzomys flavescens	21 (3.31%)	6 (2.61%)
Oxymycterus rufus	34 (5.36%)	42 (18.26%)
Monodelphis dimidiata	–	5 (2.17%)
Thylamys pallidior	–	1 (0.43%)
Species richness	7	10
Simpson index[a]	0.655 (0.68–0.725)	0.753 (0.66–0.73)
Shannon index[a]	1.305 (1.429–1.587)	1.672 (1.369–1.62)

[a] Values in parenthesis indicate 95% confidence intervals from 1000 bootstrap samples.

multiple groups in the data. The analysis removes and controls for the differences in the group means. Additionally, multiple univariate and multiple multivariate regressions for size and shape variables were used to identify robust patterns of covariation.

The morphometric and statistical analyses were carried out using MorphoJ (Klingenberg, 2011) and R 2.15.1 (R Development Core Team, 2012). The MEM analyses were performed using spacemakeR (Dray, 2012). The variation partition analysis was performed using the vegan package (Oksanen *et al*., 2011), and the analysis of correlation was done with Hmisc (Harrel, 2001). PCA and 2B-PLS analyses were carried out in MorphoJ (Klingenberg, 2011).

RESULTS

Overall, we collected 861 specimens for a total of nine sigmodontine species (Table 1). Diversity, species richness and abundances of each species by region (low and high land-use intensity) are shown in Table 1. The region with low land-use intensity tended to have greater richness and biodiversity than the region with higher land-use intensity. There were four small mammal species (*N. lasiurus*, *G. griseoflavus*, *M. dimidiata* and *T. pallidior*) that were absent from sites with higher land-use intensity. By contrast, *C. laucha* was the only species present in sites with higher land-use intensity but absent from the other sites. The abundance of common species varied in relation to site: *C. musculinus* and *C. venustus* were more abundant in sites with higher land-use intensity, whereas *A. azarae* and *O. rufus* were more representative of sites with less land-use intensity (Table 1).

MORPHOMETRIC MEASUREMENT ERROR

The percentage of measurement error (%ME), which included all sources of total variation (i.e. positioning and digitization), was two orders of magnitude smaller than intraspecific size variation, and one order of magnitude smaller than intraspecific shape variation. For mandible size, %ME ranged from 0.31 to 1.43 and from 0.29 to 1.02 for skull size. For shape, %ME ranged from 8.14 to 13.98 for mandible shape and from 8.98 to 16.6 for skull shape.

LEVELS OF VARIABILITY, ALLOMETRY AND SEXUAL DIMORPHISM

The level of intra-specific variability for the skull and mandible size showed a gradient according to habitat specialization. The coefficient of variation (CV) was higher for *C. musculinus* [skull CV = 2.59, 95% confidence interval (CI) = 2.44–2.75; mandible CV = 3.15, 95% CI = 2.97–3.35], intermediate in *A. azarae* (skull CV = 1.37, 95% CI = 1.27–1.48; mandible CV = 1.76, 95% CI = 1.61–1.91) and lower in *O. rufus* (skull CV = 1.14, 95% CI = 0.99–1.33; mandible CV = 1.61, 95% CI = 1.38–1.88). *Oxymycterus rufus* had lower levels of variation than the other two species for skull and mandible shape (Fig. S2).

Table 2. ANOVA and MANOVA for sexual dimorphism in size and shape of skull and mandible for the three species

	Size (ANOVA)			Shape (MANOVA)		
Species/structure	*F*	*P*	%var	*F*ap	*P*	%var
Cm skull	0.974	0.325	0.29	**4.181**	**< 0.0001**	**1.67**
Aa skull	**5.439**	**< 0.05**	**1.85**	**2.372**	**< 0.001**	**0.90**
Or skull	2.461	0.121	3.31	1.435	0.137	2.87
Cm mandible	**4.569**	**< 0.05**	**1.51**	**4.372**	**< 0.0001**	**1.70**
Aa mandible	0.449	0.504	0.16	**1.990**	**< 0.05**	**0.55**
Or mandible	1.613	0.208	2.19	1.476	0.169	2.11

Cm, *Calomys musculinus*; Aa, *Akodon azarae*; Or, *Oxymycterus rufus*. '%var' indicates the percentage of variation explained by sexual differences. Significant results are in bold type.

Size had a significant contribution to shape variables of mandible and skull in the three species. For *C. musculinus*, the log centroid size for skull and mandible accounted for 22% ($P < 0.0001$) and 53.65% ($P < 0.0001$) of the total shape variation, respectively. Allometry was also significant for *A. azarae*; centroid size explained 22.54% ($P < 0.0001$) and 9.19% ($P < 0.0001$) of the total shape variation for skull and mandible, respectively. Finally, the size of the structure accounted for 21.21% ($P < 0.0001$) and 7.67% ($P < 0.0001$) of the total shape variation for skulls and mandibles, respectively, in *O. rufus*.

Oxymycterus rufus did not present significant sexual dimorphism for size or shape of any of the structures (Table 2). However, *C. musculinus* and *A. azarae* presented sexual dimorphism in all traits, except for mandible size in the latter and for skull size in the former species (Table 2). Mandible size tended to be larger in females than in males for *C. musculinus*, a pattern also evident in skull size although the difference was not significant. In *A. azarae*, the skull size in males tended to be larger than in females, whereas this pattern was not significant for mandible size. When the comparisons were statistically significant, the percentage of total variation explained by sex differences did not exceed 2% (Table 2).

Overall phenotypic variation

The results of *varpart* analyses for size and shape of the two structures for the three species by sex are summarized in Tables 3 and 4. Few full models were not significant (e.g. female mandible size of *A. azarae*, and both male and female skull shape and female mandible size in *C. musculinus*). In general, much of the contribution to the full model was due to pure spatial variables. Considering the size of the skull, all the models had a significant contribution of pure spatial variables; and two of the five models of skull shape (*A. azarae* and *C. musculinus* males) did not have a significant contribution of spatial variables. For mandible size, three of the five models had a significant contribution of spatial variables (individuals of *O. rufus* and *A. azarae* and *C. musculinus* males). For mandible shape, all but one (except for *O. rufus*) of the models had a significant contribution of pure spatial contribution.

Pure climatic contribution (i.e. temperature and rainfall) was significant only in two models: skull shape of *O. rufus* and of *C. musculinus* males (Table 3). No models had an important and significant contribution of pure environmental variables on full models. The shared contributions between different sets of variables were negligible and could not be tested for significance.

Spatial phenotypic variation

Skull and mandible centroid sizes were highly correlated (*C. musculinus*: $R = 0.98$, $N = 296$; *A. azarae*: $R = 0.94$, $N = 279$; *O. rufus*: $R = 0.94$, $N = 74$; all $P < 0.001$). The skull of *O. rufus* tended to be larger in the north-eastern region of the study area than in the south-western region (high versus low land-use intensity difference in mean value, $F = 8.246$, $P < 0.01$). We found no such significant north–south differentiation in size for the other two species.

Unlike *C. musculinus* and *A. azarae*, *O. rufus* also presented a clear regional spatial pattern of shape variation, mainly differentiating the sites from the north from those from the south (Figs 2, 3). The spatial clustering in the remaining species occurred at a finer geographical scale, and appeared more marked in *A. azarae* than in *C. musculinus*, especially for skull shape. The main morphological changes in the skull involved the shape of the basicranium, with an enlargement (in *C. musculinus*) or reduction (in *A. azarae*) of the tympanic bullae, an enlargement of the foramen magnum and an anterior shift of the

Table 3. Variation partition (adjusted R^2) analyses of three sets of variables (spatial, climate and environment) on size and shape variables of skull in the three species

Skull	Entire model	Spatial//	Climate//	Environmental//	Spatial∩ Climate	Spatial∩ Environ.	Climate∩ Environ.	Shared 3
Size								
Cm ♀♀	**0.148****	**0.152****	–	–	0.0028	0.035	–	0.011
Cm ♂♂	**0.21*****	**0.152*****	–	–	0.012	0.062	0.0012	0.004
Aa ♀♀	**0.154****	**0.090****	0.008	–	–	0.085	–	–
Aa ♂♂	**0.249*****	**0.269*****	–	–	–	0.003	–	0.005
Or	**0.237***	**0.148***	–	–	0.071	0.038	0.011	0.025
Shape								
Cm ♀♀	0.0006	**0.007****	–	–	0.0002	–	0.003	–
Cm ♂♂	0.012	0.003	**0.006***	0.001	0.00001	0.0034	–	–
Aa ♀♀	**0.048*****	**0.039*****	–	–	0.004	–	0.004	0.009
Aa ♂♂	**0.028****	0.0005	0.004	0.005	0.007	0.004	0.002	0.004
Or	**0.216*****	**0.053*****	**0.015***	0.007	0.045	0.027	0.009	0.060

Cm, *Calomys musculinus*; Aa, *Akodon azarae*; Or, *Oxymycterus rufus.* The entire model considers the three sets of variables (spatial, climatic and environmental). // denotes the 'pure' effect of each set of variables when controlled for the other two sets. ∩ denotes the shared effect of two sets of variables without considering 'pure' effects of each set of variables; 'shared 3' denotes the shared effect for the three sets of variables without considering the 'pure' effects. The joint fractions cannot be tested. Significant results are in bold type. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 4. Variation partition (adjusted R^2) analyses of three set of variables (Spatial, Climate and Environment) on size and shape variables of mandible in the three species

Mandible	Entire model	Spatial//	Climate//	Environmental//	Spatial∩ Climate	Spatial∩ Environ.	Climate∩ Environ.	Shared for 3
Size								
Cm ♀♀	0.05	0.051	–	–	0.0058	0.051	0.001	–
Cm ♂♂	**0.188****	**0.128****	–	0.013	0.017	0.043	–	–
Aa ♀♀	0.014	0.037	–	–	0.0006	0.036	–	0.008
Aa ♂♂	**0.215*****	**0.233*****	–	–	–	0.022	0.002	–
Or	**0.256****	**0.192****	–	–	0.049	0.035	0.018	0.001
Shape								
Cm ♀♀	**0.042****	**0.031*****	0.005	0.016	–	–	0.0027	0.0008
Cm ♂♂	**0.03****	**0.009***	0.006	0.01	0.001	0.006	–	–
Aa ♀♀	**0.07*****	**0.022****	–	0.011	0.016	0.013	0.007	0.007
Aa ♂♂	**0.053*****	**0.038*****	0.004	0.001	0.003	0.004	0.003	0.0002
Or	**0.148*****	0.021	0.008	0.032	0.042	0.038	–	0.009

Cm, *Calomys musculinus*; Aa, *Akodon azarae*; Or, *Oxymycterus rufus.* The entire model considers the three sets of variables (spatial, climatic and environmental). // denotes the 'pure' effect of each set of variables when controlled for the other two sets. ∩ denotes the shared effect of two sets of variables without considering 'pure' effects of each set of variables and 'shared 3' denotes the shared effect for the three sets of variables without considering the 'pure' effects. The joint fractions cannot be tested. Significant results are in bold type. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

mesopterygoid fossa in the three species along PC1. Additionally, PC1 was associated with an expansion (in *A. azarae*) or a shortening (in *C. musculinus*) of the incisive foramen as well as an increase (in *A. azarae*) or a decrease (in *O. rufus*) in the upper molar tooth row length. The changes in mandible morphology were shared by the three species and included elongation or the relative position of the coronoid and angular processes. Similar patterns of morphological variation were found when individual scores were considered (Figs S3, S4).

The results of the MEM models for skull and mandible are summarized in Tables S4 and S5. Overall, we obtained better goodness of fit for size than for

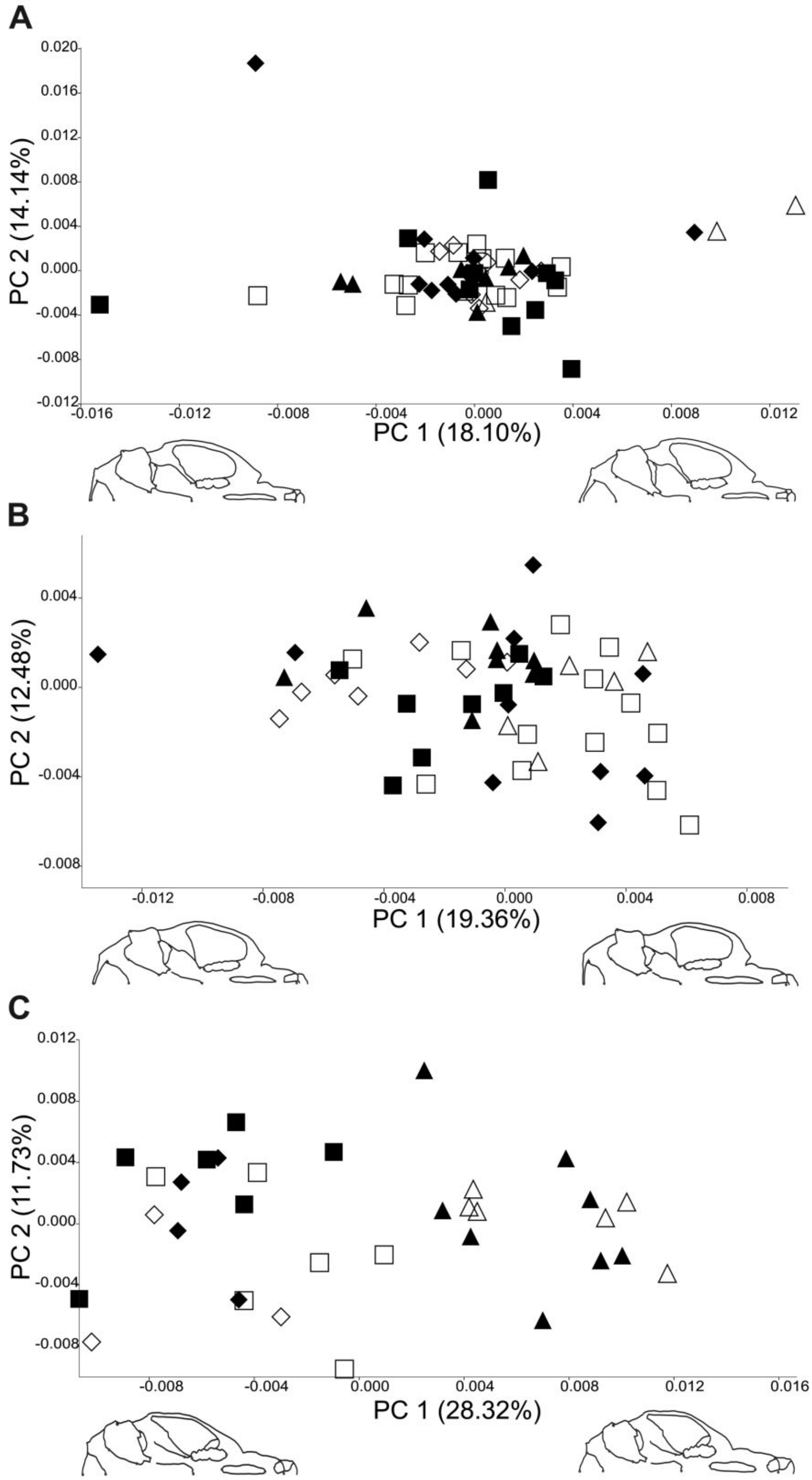

Figure 2. Skull shape differentiation among trap-lines in the three species studied: *C. musculinus* (A), *A. azarae* (B) and *O. rufus* (C). Each symbol represents the mean shape per trap-line. Different symbols are used to visualize the skull shape variation across localities according to Figure 1. Shape changes associated with the first principal component (×2) are depicted along the axis.

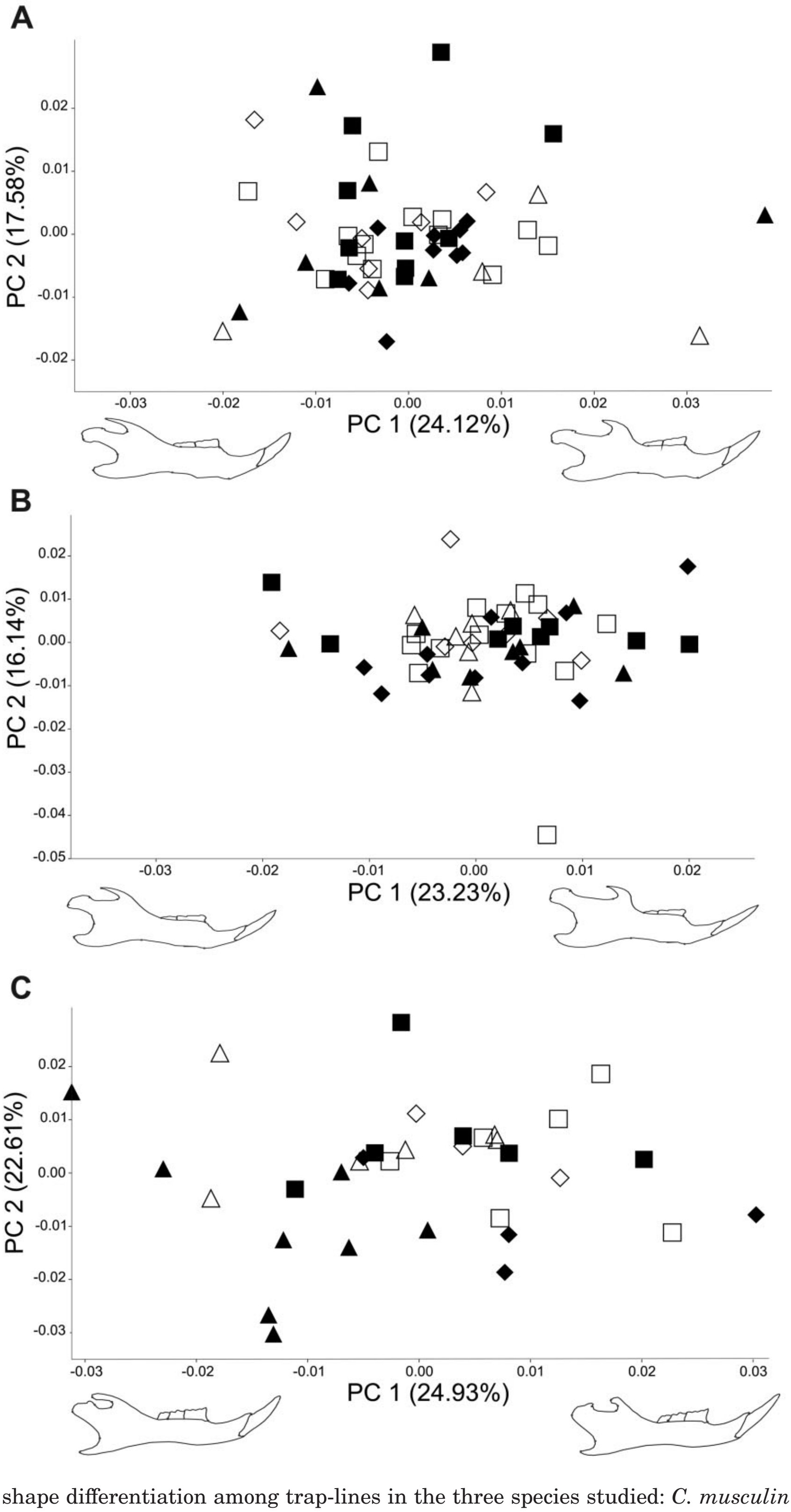

Figure 3. Mandible shape differentiation among trap-lines in the three species studied: *C. musculinus* (A), *A. azarae* (B) and *O. rufus* (C). Each symbol represents the centroid per trap-line. Different symbols are used to visualize the skull shape variation across localities according to Figure 1. Shape changes associated with the first principal component (×2) are depicted along the axis.

shape. The models explained more variance in shape variation for *O. rufus* (in both skull and mandible) than for the other two species. To a much lesser extent, the models for shape variation in skull and mandible performed better for *A. azarae* than for *C. musculinus*. Figures 4 and 5 represent the spatial patterns obtained for some of the shape variables in the three species. There was no clear geographical pattern in *C. musculinus*, but we found an east–west differentiation in skull shape for *A. azarae* (Fig. 4). There was also a north–south differentiation for spatial variation in mandible shape in *A. azarae*, especially in females, separating individuals from sites with less land-use intensity (Fig. 5). Finally, both skull and mandible shape spatial pattern indicated a north–south differentiation in *O. rufus*.

CLIMATIC AND ENVIRONMENTAL PHENOTYPIC CORRELATION

The multiple regressions of log centroid size (both for means per trap-line and for individual scores) onto the 13 environmental and climatic variables were not significant, except for the skull size of *C. musculinus* using individual scores (Table S6), indicating that skull size tends to increase with NDVI. Several regression models performed on the shape variables were statistically significant (Table 5 for trap-line means, Table S7 for regressions using individual scores). Mandible shape of *A. azarae* and *O. rufus* were significantly related to environmental and climatic variables (Table 5). In general, the independent variables explained far more variance in *O. rufus* than in the other two species. The variables mainly associated with shape variables were: environmental heterogeneity in *A. azarae* and climatic (rainfall) and environmental (heterogeneity indices and local heterogeneity variables such as BW and RSW) in *O. rufus* (Table 5). Similar results were obtained using individual shape scores (Table S7).

The results from the 2B-PLS for skull and mandible shape variables per trap-line for the three species are shown in Figures 6 and 7. For *C. musculinus* and *A. azarae*, the analyses of climatic and environmental correlation with shape were not significant (RV parameter) (Figs 6, 7). Yet, we found significant correlations in *O. rufus* (Figs 6, 7). The main morphological changes associated with these correlations were the same as described in the PCA. The climatic and environmental variables that contributed to shape correlation are summarized in Table 6. Climatic factors (i.e. rainfall and temperature) had a much stronger influence on shape variables than environmental factors. When we used individual shape scores to perform the 2B-PLS analyses, we found a gradient of correlation among the species (*C. musculinus*: skull $RV = 0.036$, mandible $RV = 0.03$, $P > 0.05$; *A. azarae*: skull $RV = 0.053$, mandible $RV = 0.049$, $P < 0.01$; *O. rufus*: skull $RV = 0.261$, mandible $RV = 0.176$, $P < 0.001$). The 2B-PLS coefficients for these analyses are given in Table S8.

DISCUSSION

In this study we assessed the spatial and environmental correlates of morphometric variation in the skull and mandible of three sigmodontine species at local scale in agroecosystems of central Argentina. Our approach included the phenotypic comparison of species with different degree of specialization in habitat use, *C. musculinus* being the most generalist species of the assemblage (Busch & Kravetz, 1992; Mills *et al*., 1992; Busch *et al*., 1997, 2000; Ellis *et al*., 1998), followed by *A. azarae*, which could be considered intermediate in its degree of habitat use specialization (Polop, 1996; Suárez & Bonaventura, 2001), and finally by *O. rufus*, which is considered the most specialized due to its semi-fossorial habits.

SEXUAL DIMORPHISM

We first analysed the pattern of sexual dimorphism in size and shape for skull and mandible of the three species. Cardini & Elton (2008) considered that sexual phenotypic dimorphism in general, and sexual size dimorphism in particular, could be related to mating system, diet, phylogenetic inertia and/or niche differentiation. It is well established that sexual selection could cause sexual size dimorphism (Andersson, 1994), and our results could be explained, at least in part, in light of the differences in mating systems, indicating that sexual selection may be acting in *A. azarae*. Recently, Bonatto *et al*. (2012, 2013) found evidence for a polygynous mating system in *A. azarae*. Males have larger home-ranges than females, and their home-ranges overlap largely whereas there is little home-range overlap between males. Body size in this species is also biased towards males (Bonatto *et al*., 2012; 2013), in accordance with our results for skull and mandible size. On the other hand, studies by Steinmann *et al*. (2005) and Steinmann, Priotto & Polop (2009) indicate that *C. musculinus* has a promiscuous mating system. Accordingly, we found no clear evidence for sexual size dimorphism in *C. musculinus*. There is no study inferring the mating system in *O. rufus*. Contrary to our findings, Suárez, Cueto & Kravetz (1998) reported the presence of sexual dimorphism in cranial measurements for this species, with males being longer than females, and this difference was greater than for body size sexual dimorphism.

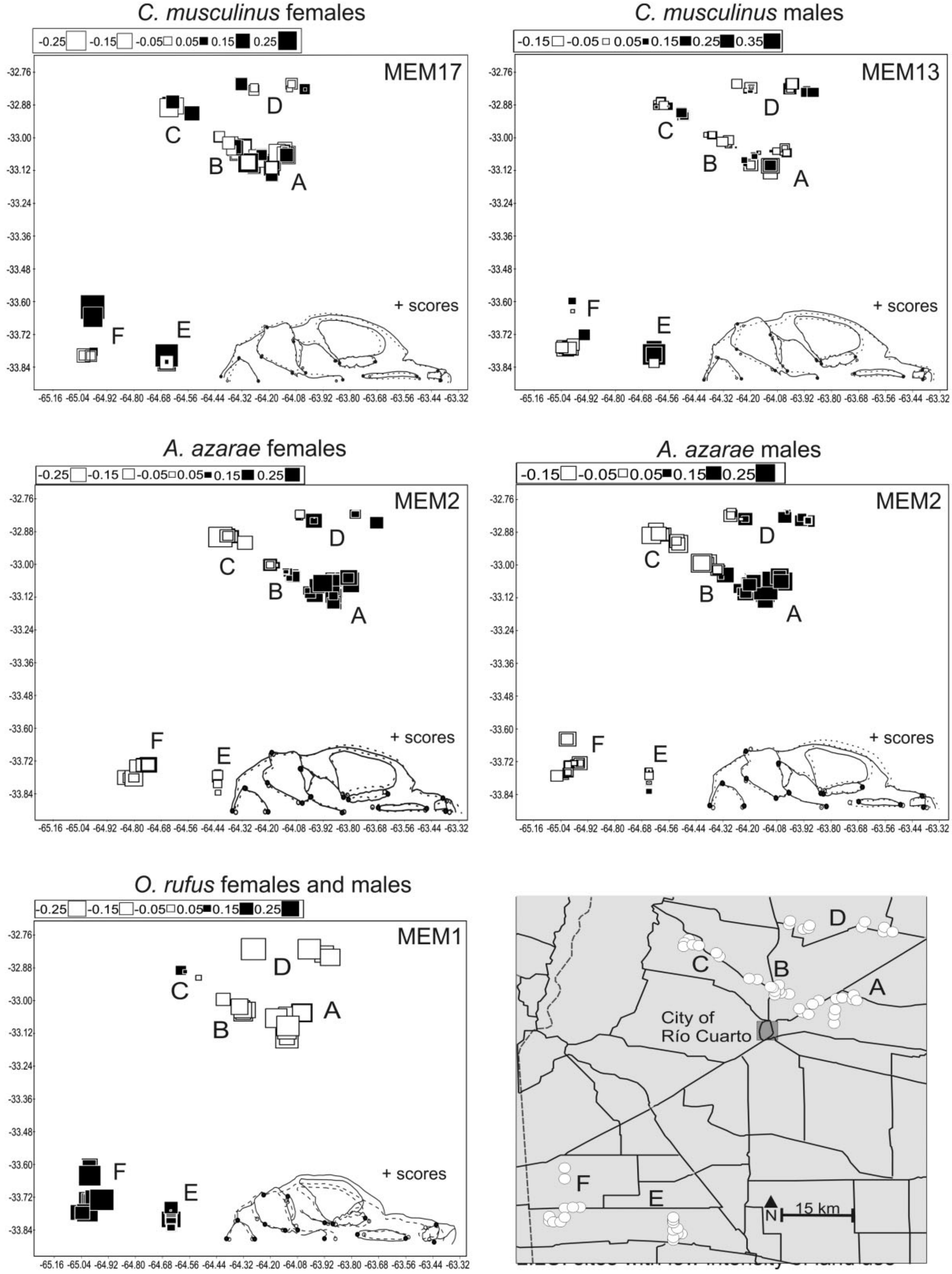

Figure 4. Spatial patterns of skull shape variation obtained by the Moran eigenvector map (MEM) method: x-axis, latitude; y-axis, longitude. For each species (and sex), the most representative and significant (positive spatial autocorrelation) MEM variable is depicted. Black squares represent positive values and white squares negative values. The size of the squares is related to the associated value. Shape changes (×2) obtained by linear regression associated with the increasing MEM values are also depicted. Sites with high land-use intensity: A, B, C and D; sites with low land-use intensity: E and F.

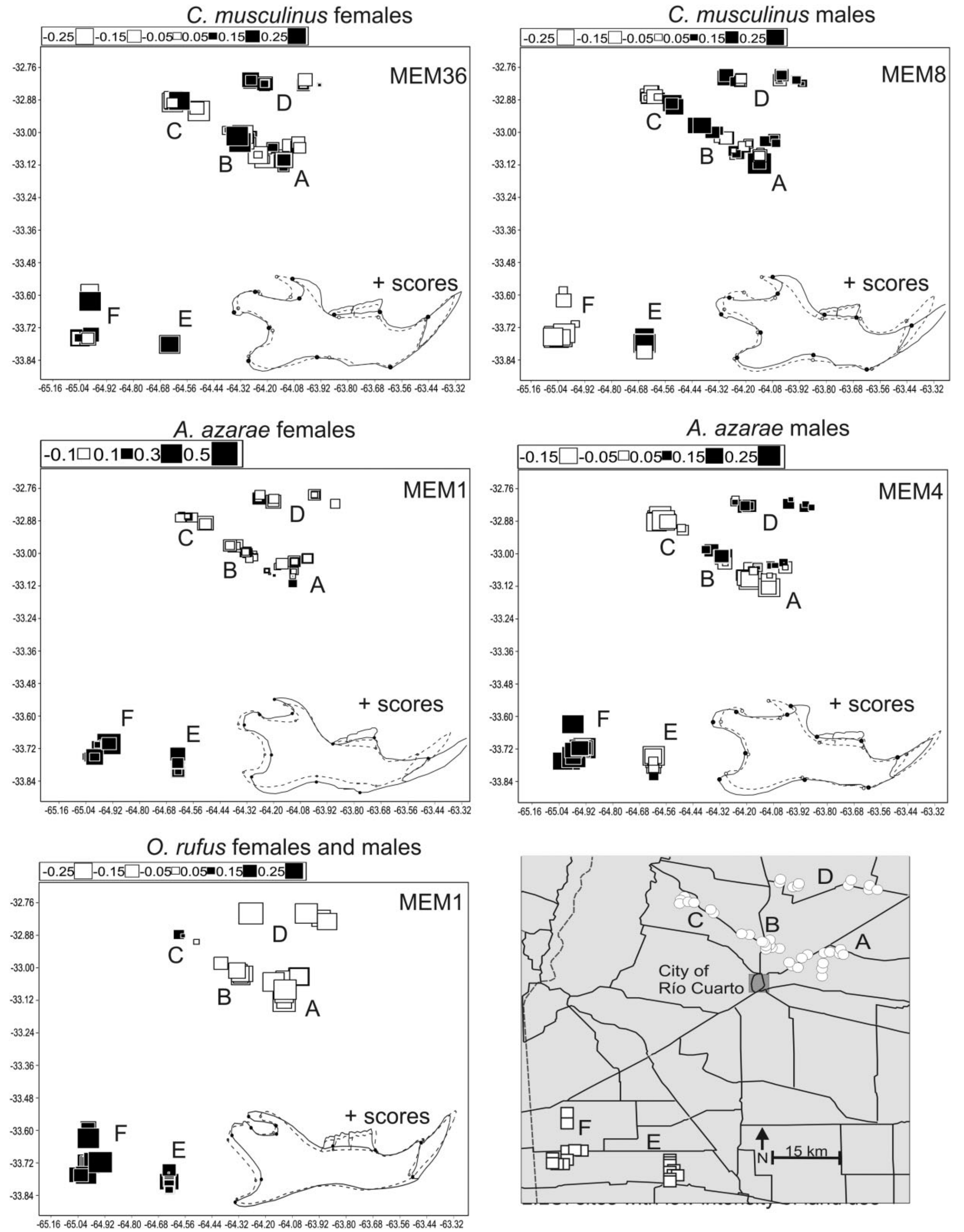

Figure 5. Spatial patterns of mandible shape variation obtained by the Moran eigenvector map (MEM) method. For each species (and sex) the most representative and significant (positive spatial autocorrelation) MEM variable is depicted. Black squares represent positive values and white squares negative values. The size of the squares is related to the associated value. Shape changes (×2) obtained by linear regression associated with the increasing MEM values are also depicted. Sites with high land-use intensity: A, B, C and D; sites with low land-use intensity: E and F.

Table 5. Coefficients of the multivariate multiple regression of shape (skull and mandible) per trap-line onto 13 environmental and climatic variables for the three species studied

	C. musculinus				*A. azarae*				*O. rufus*			
Shape	Skull ($N = 50$)		Mandible ($N = 49$)		Skull ($N = 51$)		Mandible ($N = 51$)		Skull ($N = 33$)		Mandible ($N = 33$)	
P model	0.392		0.927		0.278		***0.034*****		0.268		***0.041****	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
PC%var	18.10	14.14	24.12	17.58	19.36	12.48	23.23	16.14	28.32	11.73	24.93	22.61
R^2	0.139	0.299	0.416	0.265	***0.437****	0.345	0.261	0.264	***0.762*****	0.325	0.561	***0.651****
BH	0.000	0.001	0.002	0.002	0.000	–0.001	0.000	0.000	0.001	0.000	0.002	0.001
BW	0.000	–0.001	0.003	–0.001	***0.002*****	0.000	0.000	0.000	0.000	–0.001	–0.004	***0.008*****
RSW	0.002	–0.001	–0.001	0.000	–0.001	0.001	0.000	0.001	0.000	–0.002	0.002	***0.009*****
HI2	0.000	0.000	***–0.004****	–0.001	0.000	0.001	–0.003	–0.003	0.001	0.000	0.003	0.002
HI3	0.000	0.000	–0.002	–0.003	0.000	0.000	0.004	***–0.004****	–0.001	–0.002	0.001	0.004
HI4	0.000	0.000	0.001	0.002	–0.001	0.000	–0.001	0.002	0.000	0.000	–0.005	–0.003
HI5	0.000	0.001	–0.003	0.001	***0.002*****	–0.001	0.001	0.003	0.002	–0.001	–0.007	0.004
HI7	0.001	–0.001	–0.003	0.000	0.001	0.000	–0.001	0.003	***0.003****	0.001	0.001	–0.003
HI9	0.000	0.000	0.003	–0.003	***–0.002****	0.000	–0.003	–0.002	–0.002	0.001	0.006	***–0.008****
HI10	–0.001	0.000	0.003	–0.001	–0.002	0.000	0.001	0.000	–0.002	0.002	0.002	–0.005
NDVI	0.000	–0.001	0.001	–0.001	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.002
Temp	–0.001	–0.001	–0.003	***0.005****	–0.001	0.001	–0.002	0.002	0.001	0.000	–0.004	0.006
Rainf	0.001	0.001	0.001	0.001	0.000	0.001	0.002	–0.001	***0.004*****	–0.001	–0.003	–0.001

Principal components were utilized as shape variables due to their uncorrelated nature. 'P model' correspond to the full multivariate multiple regression. 'PC%var' indicates the percentage of variation accounted for in the first two principal components considered. The amount of explained variance by the independent variables is depicted (R^2). Values in bold italics indicate significant statistical value for the coefficient of regression.

*$P < 0.05$, **$P < 0.01$. BH, border height; BW, border width; HI(2–5,7,9–10), heterogeneity indices; NDVI, normalized difference vegetation index; Rainf, rainfall; RSW, road side width; Temp, temperature.

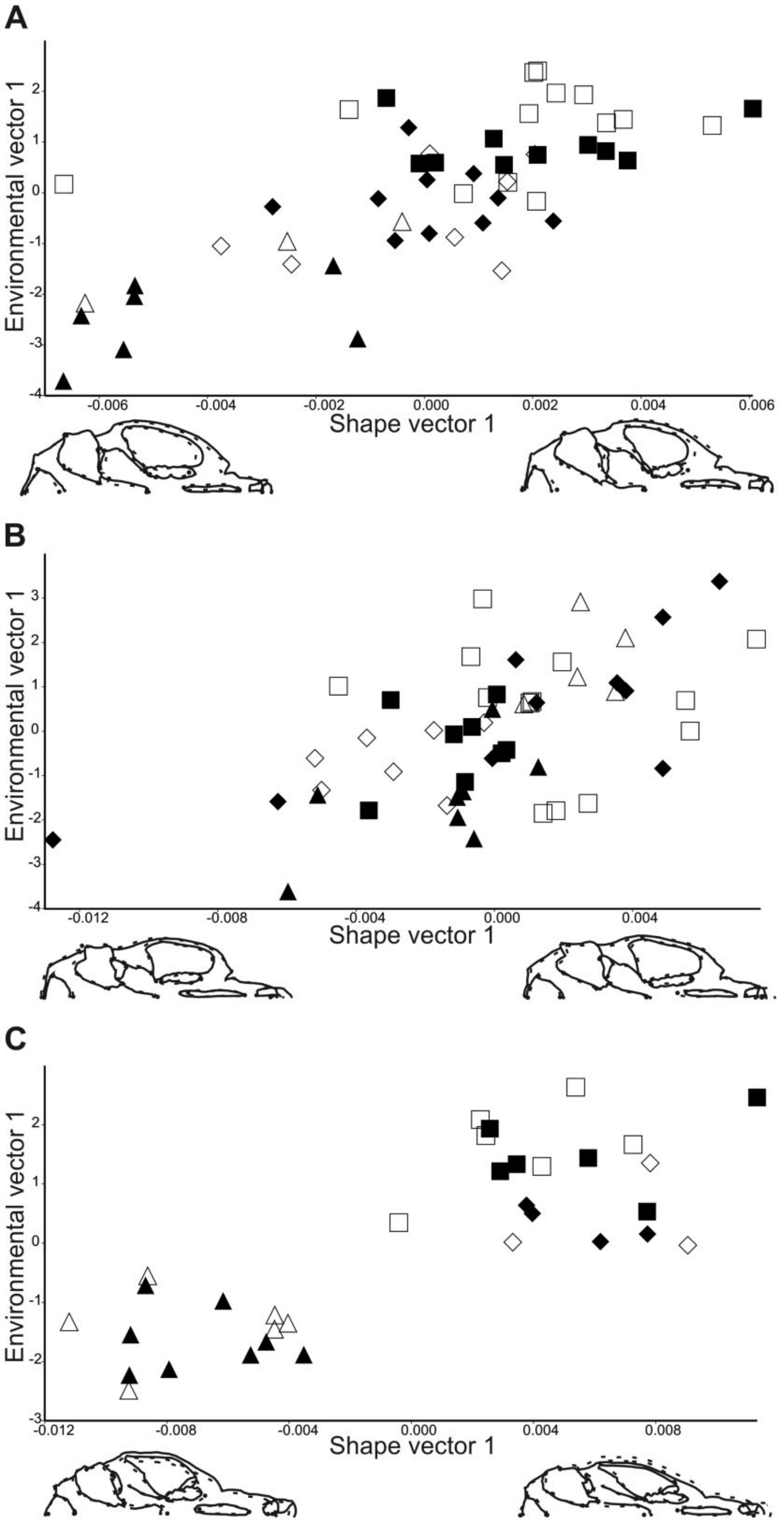

Figure 6. Two block-partial least square (2B-PLS) results for skull mean shape variation and climatic and environmental variables per trap-line for the three species. A, *C. musculinus*; B, *A. azarae*; C, *O. rufus*; *x*-axis, shape change; *y*-axis, climatic and environmental change. Shape changes (continuous lines) with respect to consensus shape (dashed lines) for the negative and positive ends of the shape vector are shown. Symbols represent trap-lines according to Figure 1.

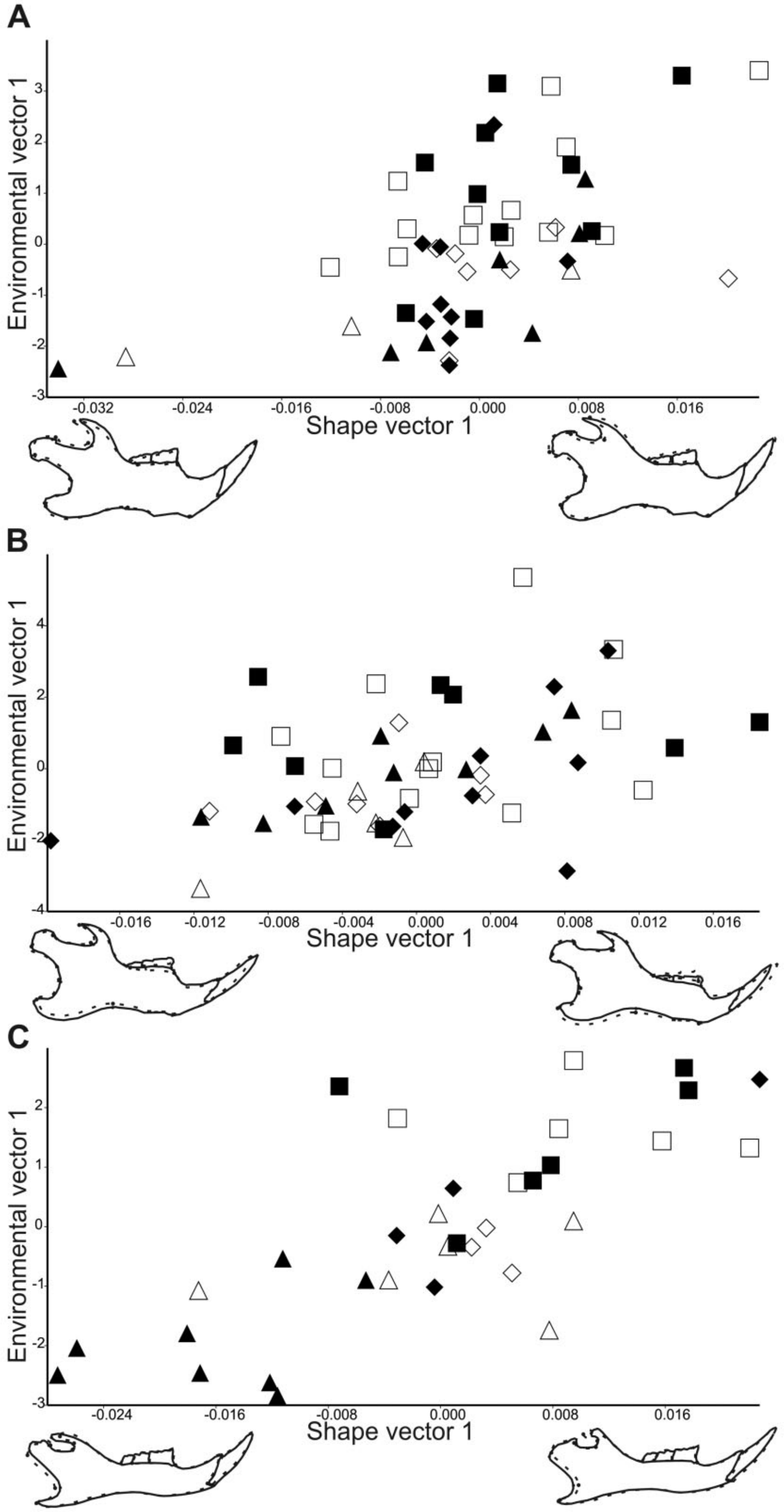

Figure 7. Two block-partial least square (2B-PLS) results for mandible mean shape variation and climatic and environmental variables per trap-line for the three species. A, *C. musculinus*; B, *A. azarae*; C, *O. rufus*. *x*-axis, shape change; *y*-axis, climatic and environmental change. Shape changes (continuous lines) with respect to consensus shape (dashed lines) for the negative and positive ends of the shape vector are shown. Symbols represent trap-lines according to Figure 1.

Table 6. Two blocks-partial least squares coefficients and percentage of covariation (Covar%) between skull and mandible mean shape per trap-line and 13 environmental and climatic variables for the three species studied

	C. musculinus				*A. azarae*				*O. rufus*			
	Skull		Mandible		Skull		Mandible		Skull		Mandible	
	PLS1	PLS2	PLS1	PLS2	PLS1	PLS2	PLS1	PLS2	PLS1	PLS2	PLS1	PLS2
Covar %	36.38	22.70	43.97	20.39	39.62	21.85	26.50	24.98	63.01	15.31	54.89	16.16
P	0.208	0.100	0.213	0.357	0.139	0.169	0.928	0.329	**0.003**	0.322	**0.014**	0.382
BH	***0.288***	***–0.303***	0.155	***–0.415***	0.146	***–0.461***	0.197	–0.221	0.119	–0.303	***0.322***	–0.077
BW	–0.062	0.239	***0.363***	0.202	0.193	–0.045	***0.322***	0.161	0.238	–0.115	0.279	***0.416***
RSW	***0.362***	–0.179	***0.392***	–0.149	0.063	–0.204	0.029	0.075	***0.328***	–0.214	***0.508***	0.144
HI2	–0.061	***0.423***	***0.396***	***0.302***	***–0.342***	–0.142	***0.527***	0.289	–0.088	–0.311	0.135	***–0.372***
HI3	–0.089	0.139	0.027	0.215	***–0.396***	–0.074	0.169	***–0.542***	0.172	***–0.397***	–0.037	–0.272
HI4	0.037	***0.357***	0.294	0.238	***–0.459***	***–0.293***	***0.364***	–0.007	0.04	***–0.404***	0.035	***–0.395***
HI5	–0.179	–0.276	0.218	0.178	0.213	–0.019	0.166	***0.388***	***–0.257***	–0.157	–0.084	0.143
HI7	–0.097	0.179	–0.129	0.173	–0.328	0.047	0.234	–0.141	–0.073	***–0.354***	–0.136	–0.249
HI9	–0.246	–0.269	–0.114	0.127	–0.124	0.213	***0.395***	0.23	–0.115	***–0.343***	0.059	***–0.369***
HI10	–0.143	***0.373***	***0.433***	0.279	***–0.413***	–0.171	0.267	–0.155	0.127	***–0.359***	0.049	0.095
NDVI	0.021	***0.308***	–0.006	0.075	–0.074	–0.132	–0.067	***0.376***	–0.187	0.029	0.086	–0.337
Temp	***–0.544***	0.044	–0.291	***0.515***	***–0.325***	***0.413***	–0.222	0.217	***–0.363***	–0.007	***–0.468***	0.287
Rainf	***–0.591***	–0.278	***–0.312***	***0.380***	–0.039	***0.606***	–0.234	***0.326***	***–0.718***	–0.186	***–0.53***	–0.092

Main coefficients related to the first two partial least squares axes are in italics. BH, border height; BW, border width; HI(2–5,7,9–10), heterogeneity indices; NDVI, normalized difference vegetation index; Rainf, rainfall; RSW, roadside width; Temp, temperature.

EFFECT OF LAND USE ON MORPHOLOGICAL VARIATION

Our hypothesis was that the intensity of land-use change would affect morphological variation across our studied sites, and that this effect would vary across species, depending on their degree of habitat specialization. *Calomys musculinus* and *A. azarae* are the most abundant rodent species inhabiting the Argentine agroecosystems and presented the highest level of variability among the three species studied for the size and shape of the skull and mandible). *Akodo azarae* uses several types of habitats but is dominant in more stable ones such as native grasslands, railway roads, fence lines and border lines (i.e. crop field edges) (Polop, 1996; Suárez & Bonaventura, 2001; Gomez *et al*., 2011). *Calomys musculinus* not only uses similar habitats to *A. azarae* but also thrives in highly modified habitats and has a wider habitat and trophic niche than other coexisting rodent species in agrarian systems of central Argentina (Busch *et al*., 2000). Our third study species, *O. rufus*, presented the lowest level of variability for size and shape, and changes its habitat use with the season: it exploits herbaceous and grassland roadsides during the autumn, whereas it is almost exclusively captured in habitats with dense tree stratum during the summer and winter (Suárez, 1994). Seasonal variations in habitat use by *O. rufus* could be related to temperature restrictions, and Suárez (1994) thus describes it as a temperature-sensitive species. Yet, in our study, we detected a strong morphological structure in *O. rufus*, which was related to environmental and habitat variables measured over one season only. This result suggests that seasonality in habitat use is not as strong as the overall environmental and habitat variation across our study area.

We also assessed the spatial phenotypic variation in relation to habitat heterogeneity. We divided the sites according to the intensity of land use following the socioeconomic information of Cisneros *et al*. (2008), who showed that the province suffered intensive habitat modification in central regions due to agricultural practices, whereas the south-west, largely composed of remnants of the Espinal forest and natural pastures for cattle, suffered less agricultural activities. Pergams & Lawler (2009) found that several morphometric traits associated with body size in rodents from the US presented a positive trend with changes in precipitation and human population density. As human population density increases, so do the quality and abundance of rodent food resources, allowing rodent species to grow larger. Our results indicate that *O. rufus* tends to be larger in the north-eastern part of our study area, the area surrounding the city of Río Cuarto (160 000 inhabitants) with high-intensity land use and increased food resources for the rodents. The effects of human population density on the size and morphological variation in wildlife need to be analysed further.

ENVIRONMENTAL FACTORS AND PHENOTYPIC VARIATION

Beside a local plastic response to increased food supply, the pattern of variation in size we observed could also reflect a more regional pattern of body size increase from south to north in response to environmental gradients such as Bergmann's rule (reviews in Ashton, Tracy & de Queiroz, 2000; Millien *et al*., 2006; Teplitsky & Millien, 2014). In our study area, there is congruence between latitudinal climatic variation and land-use intensity.

Using Moran eigenvector maps to describe spatial patterns of size and shape variation, we found that, in general, size variables had a better fit with spatial variables than shape variables in the three analysed species. The high plasticity and adaptive nature of size is well documented (Cardini, Jansson & Elton, 2007). However, the strength of the relative effects of geographical and climatic conditions on size and shape variation remains of debate. Monteiro, Duarte & dos Reis (2003) found a correlation between skull shape and environmental gradients in the Brazilian echimyd rodent *Thrichomys apereoides* but skull size did not follow this same pattern. A similar result was reported for one of the two field mouse species of the genus *Apodemus* from the Japanese archipelago (Renaud & Millien, 2001; Millien, 2004). Renaud & Michaux (2003) found significant correlation between mandible shape and latitude but not between mandible size and latitude in the European *Apodemus sylvaticus*.

Finally, while specialist species may mostly be affected by environmental stressors such as habitat fragmentation, parasites or chemicals utilized in agroecosystems, some generalist species may benefit from competition relaxation in these systems. We thus conclude that populations of specialist species such as *O. rufus* may suffer more from environmental stress than generalist species. In their study of morphological variation in two coexisting species of wood mice *Apodemus* from Japan, Renaud & Millien (2001) pointed out that different ecological preferences among the species might explain their difference in response to the similar environmental variation. Interspecific competition has previously been shown as a possible driver of skull and mandible phenotypic variation through community-wide character displacement (e.g. Yom-Tov, 1991; Dayan & Simberloff, 1994; Parra, Loreau & Jaeger, 1999; Millien-Parra & Loreau, 2000; Ledevin *et al*., 2012). Evidence for competition among the sigmodontine species from the

Pampean region comes from the observed inverse abundances between *A. azarae* and *C. musculinus* (Crespo, 1966) or habitat selection studies (Busch *et al*., 1997). The differences in habitat use among species were also attributed to interspecific competition, *A. azarae* being dominant over species of *Calomys* (Kravetz & de Villafañe, 1981; Kravetz & Polop, 1983). Unfortunately, *O. rufus* was not considered in previous studies of habitat selection in the Pampean region. Dellafiore & Polop (2010) suggested that food-related interspecific competition among sigmodontines from central Argentina may be a mechanism for species morphological differentiation. Castellarini, Dellafiore & Polop (2003) further showed that dietary overlap is higher in highly disturbed habitats. Future studies testing the effect of interspecific competition on phenotypic variation should help to shed light on these questions.

Patterns of geographical structure

Understanding the genetic differences among populations may allow us to infer if these patterns of morphological variation are due to phenotypic plasticity (or ecophenotypy, Caumul & Polly, 2005) that is not driven by natural selection (adaptation to the environment). Ongoing genetic studies may clarify this aspect. Additionally, information about gene flow and dispersal are also relevant to understanding the morphological patterns of differentiation in changing environments (e.g. Ledevin & Millien, 2013). Pergams & Lacy (2007) suggested that replacement of regional populations with immigrants from genetically distinct neighbouring populations facilitated by environmental changes can explain the rapid morphological change in a local population of *Peromyscus leucopus*.

In our study system, there is a significant geographical structure for the three studied species (even for the generalist species) that seems to vary at a very fine scale. L. Sommaro *et al*. (unpubl. data), using microsatellite loci, showed that individuals of *C. musculinus* presented a positive spatial autocorrelation at a scale as small as 300 m, this pattern being more marked in females than in males. A similar pattern of spatial autocorrelation at a fine geographical scale is apparent at the molecular level in *A. azarae* (N. Vera, pers. communication). Moreover, *A. azarae* displays an intriguing east-west pattern of shape variation. This pattern could be related to the presence of both natural and artificial geographical barriers to dispersal as revealed by microsatellite markers (N. Vera, personal comm.). *Oxymycterus rufus* presented a strong spatial structure mainly along a north–south direction. However, there are no genetic data available in the area to support our results.

Several phylogeographical and population genetic studies performed in *C. musculinus* from central Argentina concluded that this species underwent a recent demographic and geographical expansion, with low to moderate gene flow among populations (González-Ittig, Patton & Gardenal, 2007). Besides, Chiappero *et al*. (2010) studied the genetic differentiation among populations in two types of altered habitats: the city of Rio Cuarto and its surrounding agroecosystem, very close to our study system. Rural populations presented lower genetic differentiation than those inhabiting an urban landscape. In a phylogeographical study at a regional scale, Trimarchi (2012) showed that populations of *A. azarae* presented a high genetic differentiation between them and the magnitude of such differentiation was correlated with geographical distance (i.e. a significant isolation-by-distance genetic pattern). Unfortunately, population genetics studies on *O. rufus* are not abundant, which could allow us to make some predictions on the environmental–morphometric differentiation and patterns of genetic differences over the species distribution. A single genetic study involving *O. rufus* samples was reported by Gonçalves & de Oliveira (2004). The authors found that Argentine samples diverged genetically by 0.1–1.2%. Considering the habitat specialization of this species we predict that genetic structure will be stronger and at finer scale than in the other two species. Further population genetic studies are needed to understand the geographical patterns of differentiation in *O. rufus* samples from central Argentina.

CONCLUSIONS

In our study system, climatic variables (i.e. temperature and rainfall) were always correlated with shape variation, whereas only a few variables related to environmental heterogeneity were correlated with skull and mandible shape variation. Debat, Debelle & Dworkin (2009) pointed out that the complexity of developmental regulation appears to make shape variables resilient to rapidly changing environments. Perhaps environmental and habitat heterogeneity caused by agriculture practices are too recent to influence phenotypic variation in comparison with climatic factors. We also found that size is less influenced by climatic and environmental variables than shape. Accordingly, Breuker, Patterson & Klingenberg (2008) studied the developmental buffering of wing shape in different *Drosophila* genotypic strains and concluded that the developmental links between size and shape are weak and that various morphological traits can respond differently to external factors.

Our results confirm that the strength of the effects of environmental variables and land-use intensity on the phenotype of *C. musculinus*, *A. azarae* and *O. rufus* is conditioned, at least in part, by their degree of specialization in habitat use. Specialist species, such as *O. rufus* and *A. azarae*, displayed more differentiation and a stronger spatial structure than *C. musculinus*, a more generalist species.

To conclude, our study provides evidence for the value of an integrative approach to better understand the spatial pattern of phenotypic variation and its drivers, from climatic, environmental habitat and land-use factors as well as species interactions and ecological specialization. These factors are all jointly affecting species dynamics and local persistence in changing environments, and their effects and mechanisms need to be integrated if we are to improve our efforts for biodiversity conservation.

ACKNOWLEDGEMENTS

We are grateful to members of the GIEPCO lab for fieldwork assistance, especially Lucia Sommaro for skull preparation and Daniela Gomez for her comments and corrections to the English text. Thanks to all members of the Millien lab for hospitality and help with data analyses. Thanks to Jorge Gaitán and Robby Marrotte who helped with different aspects of this project, and to Rodrigo Lima whose comments improved earlier versions of the manuscript. Thanks to Noelia Vera for sharing her preliminary results with us. We thank the three anonymous reviewers for their helpful comments. This work was supported by a grant 11220100100003 of CONICET to J.W.P. and a NSERC discovery grant #341918 to V.M. The authors declare that there is no conflict of interest.

REFERENCES

Andersson M. 1994. *Sexual selection*. Princeton, NJ: Princeton University Press.

Ashton KG, Tracy MC, de Queiroz A. 2000. Is Bergmann's rule valid for mammals? *American Naturalist* **156:** 390–415.

Baldi G, Guerschman JP, Paruelo JM. 2006. Characterizing fragmentation in temperate South America grasslands. *Agriculture, Ecosystems and Environment* **116:** 197–208.

Baldi G, Paruelo JM. 2008. Land-use and land cover dynamics in South America temperate grasslands. *Ecology and Society* **13:** 6.

Barlow JC. 1969. Observations of the biology of rodents in Uruguay. *Life Sciences Contributions Royal Ontario Museum* **75:** 1–59.

Bertin A, Ruíz VH, Figueroa R, Gouin N. 2012. The role of spatial processes and environmental determinants in microgeographic shell variation of the freshwater snail *Chilina dombeyana* (Bruguière, 1789). *Naturwissenchaften* **99:** 225–232.

Bilenca DN, González-Fischer CM, Teta P, Zamero M. 2007. Agricultural intensification and small mammal assemblages in agroecosystems of the Rolling Pampas, central Argentina. *Agriculture, Ecosystems and Environment* **121:** 371–375.

Bilenca DN, Kravetz FO. 1995. Daño a maíz por roedores en la región Pampeana (Argentina), y un plan para su control. *Vida Silvestre Neotropical* **4:** 51–57.

Blanchet FG, Legendre P, Borcard D. 2008. Modelling directional spatial processes in ecological data. *Ecological Modelling* **215:** 325–336.

Blanchet FG, Legendre P, Maranger R, Monti D, Pepin P. 2011. Modelling the effect of directional spatial ecological processes at different scales. *Oecologia* **166:** 357–368.

Bonatto F, Coda J, Gomez D, Priotto J, Steinmann A. 2013. Inter-male aggression with regard to polygynous mating system in Pampean grassland mouse, *Akodon azarae* (Cricetidae: Sigmodontinae). *Journal of Ethology* **31:** 223–231.

Bonatto F, Gomez D, Steinmann A, Priotto J. 2012. Mating strategies of the Pampean mouse males. *Animal Biology* **62:** 381–396.

Bookstein FL. 1991. *Morphometric tools for landmark data: geometry and biology*. New York: Cambridge University Press.

Borcard D, Gillet F, Legendre P. 2010. *Numerical ecology with R*. New York: Springer.

Breuker C, Patterson JS, Klingenberg CP. 2008. A single basis for developmental buffering of *Drosophila* wing shape. *PLoS One* **1:** e7.

Burkart R, Bárbaro NO, Sánchez RO, Gómez DA. 1999. *Eco-regiones de la Argentina*. Administración de Parques Nacionales, Programa de Desarrollo Institucional Ambiental, Buenos Aires. 42.

Busch M, Alvarez MR, Cittadino EA, Kravetz FO. 1997. Habitat selection and interspecific competition in rodents in pampean agroecosystems. *Mammalia* **61:** 167–184.

Busch M, Kravetz FO. 1992. Competitive interactions among rodents (*Akodon azarae*, *Calomys laucha*, *Calomys musculinus* and *Oligoryzomys flavescens*) in two habitat systems. I. Spatial and numerical relationships. *Mammalia* **56:** 45–56.

Busch M, Miño MH, Dadon JR, Hodara K. 2000. Habitat selection by *Calomys musculinus* (Muridae, Sigmodontinae) in crop areas of the pampean region, Argentina. *Ecologia Austral* **10:** 15–26.

Cardini A, Elton S. 2008. Variation in guenon skulls (II): sexual dimorphism. *Journal of Human Evolution* **54:** 638–647.

Cardini A, Jansson A-U, Elton S. 2007. A geometric morphometric approach to the study of ecogeographical and clinal variation in vervet monkeys. *Journal of Biogeography* **34:** 1663–1678.

Castellarini F, Dellafiore C, Polop J. 2003. Feeding habits of small mammals in agroecosystems of central Argentina. *Mammalian Biology* **68:** 91–101.

Caumul R, Polly PD. 2005. Phylogenetic and environmental components of morphological variation: skull, mandible and molar shape in marmots (*Marmota*, Rodentia). *Evolution* **59:** 2460–2472.

Cavia R, Gomez Villafañe IE, Cittadino EA, Bilenca DN, Miño MH, Busch M. 2005. Effects of cereal harvest on abundance and spatial distribution of the rodent *Akodon azarae* in central Argentina. *Agriculture Ecosystems and Environment* **107:** 95–99.

Chiappero MB, Panzetta-Dutari GM, Gómez D, Castillo E, Polop JJ, Gardenal CN. 2010. Contrasting genetic structure of urban and rural populations of the wild rodent *Calomys musculinus* (Cricetidae, Sigmodontinae). *Mammalian Biology* **76:** 41–50.

Cisneros JM, Cantero A, Degioanni A, Becerra VH, Zubrzycki MA. 2008. Producción, uso y manejo de las tierras. In: de Prada JD, Penna J, eds. *Percepción económica y visión de los productores agropecuarios de los problemas ambientales en el sur de Córdoba, Argentina*. Buenos Aires: Publicaciones Nacionales INTA, 31–44.

Colangelo P, Castiglia R, Franchini P, Solano E. 2010. Pattern of shape variation in the eastern African gerbils of the genus *Gerbilliscus* (Rodentia, Muridae): environmental correlations and implication for taxonomy and systematic. *Mammalian Biology* **75:** 302–310.

Crespo JA. 1966. Ecología de una comunidad de roedores silvestres en el Partido de Rojas, Provincia de Buenos Aires. *Revista Museo Argentino de Ciencias e Instituto Nacional de Investigación en Ciencias Naturales, Ecología* **1:** 79–134.

Dalby PL. 1975. Biology of Pampa rodents, Balcarce area, Argentina. *Publications of the Museum of Michigan State University, Biological Series* **5:** 149–271.

Dayan T, Simberloff D. 1994. Morphological relationships among coexisting heteromyids: an incisive dental character. *American Naturalist* **143:** 462–477.

Dayan T, Wool D, Simberloff D. 2002. Variation and covariation of skull and teeth: modern carnivores and the interpretation of fossil mammals. *Paleobiology* **28:** 508–526.

Debat V, Debelle A, Dworkin I. 2009. Plasticity, canalization, and developmental stability of the *Drosophila* wing: joint effects of mutations and developmental temperature. *Evolution* **63:** 2864–2876.

Dellafiore CM, Polop JJ. 2010. La alimentación en los sigmodontinos de la región central de Argentina. In: Polop JJ, Busch M, eds. *Biología y Ecología de pequeños roedores en la región pampeana de Argentina. Enfoques y perspectivas*. Córdoba: Universidad Nacional de Córdoba, 173–199.

Devictor V, Clavel J, Julliard R, Lavergne S, Mouillot D, Thuiller W, Venail P, Villéger S, Mouquet N. 2010. Defining and measuring ecological specialization. *Journal of Applied Ecology* **47:** 15–25.

Dray S. 2012. *spacemakeR: spatial modelling*. R package. Available at: http://r-forge.r-project.org/R/?group_id=195 [Accessed July 2012].

Dray S, Legendre P, Peres-Neto PR. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbor matrices (PCNM). *Ecological Modelling* **196:** 483–493.

Dujardin JP. 2008. Morphometrics applied to medical entomology. *Infection, Genetics and Evolution* **8:** 875–890.

Ellis BA, Mills JN, Glass GE, McKee KT, Enria DA, Childs JE. 1998. Dietary habits of the common rodents in agroecosystems in Argentina. *Journal of Mammalogy* **79:** 1203–1220.

Fraschina J, León VA, Busch M. 2012. Long-term variations in rodent abundance in a rural landscape of the Pampas, Argentina. *Ecolological Research* **27:** 191–202.

García-Gigorro S, Saura S. 2005. Forest fragmentation estimated from remotely sensed data: is comparison across scale possible? *Forest Science* **51:** 51–63.

Gomez D, Sommaro L, Steinmann A, Chiappero M, Priotto J. 2011. Movement distances of two species of sympatric rodents in linear habitats of Central Argentine agro-ecosystems. *Mammalian Biology* **76:** 58–63.

Gonçalves PR, de Oliveira JA. 2004. Morphological and genetic variation between two sympatric forms of *Oxymycterus* (Rodentia, Sigmodontinae): an evaluation of hypotheses of differentiation within the genus. *Journal of Mammalogy* **85:** 148–161.

González-Ittig RE, Patton JL, Gardenal CN. 2007. Analysis of cytochrome-b nucleotide diversity confirms a recent range expansion in *Calomys musculinus* (Rodentia, Muridae). *Journal of Mammalogy* **88:** 777–783.

Grinnell J. 1917. The niche relationship of the California Thrasher. *The Auk* **34:** 427–433.

Hall AJ, Rebella CM, Ghersa CM, Culot JP. 1992. Field crop systems of the Pampas. In: Pearson CJ, ed. *Ecosystems of the world, field crop ecosystems*. Amsterdam: Elsevier, 413–450.

Harrel FE. 2001. *Hmisc*. R package. Available at: http://cran.r-project.org/web/packages/Hmisc/index.html [Accessed July 2012].

Klingenberg CP. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* **11:** 353–357.

Klingenberg CP, McIntyre GS. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. *Evolution* **52:** 1363–1375.

Kravetz FO. 1972. Estudio del régimen alimentario, períodos de actividad y otros rasgos ecológicos en una población de ‘ratón hocicudo’ (*Oxymycterus rufus platensis*, Thomas) de Punta Lara. *Acta Zoologica Lilloana* **29:** 201–212.

Kravetz FO, Polop JJ. 1983. Comunidades de roedores en agroecosistemas del Departamento Río Cuarto. *Ecosur, Argentina* **10:** 1–18.

Kravetz FO, de Villafañe G. 1981. Poblaciones de roedores en cultivo de maíz durante etapas de madurez y rastrojo. *Historia Natural* **1:** 213–232.

Lalis A, Baylac M, Cosson JF, Makundi RH, Machang’u RS, Denys C. 2009. Cranial morphometric and fine scale genetic variability of two adjacent *Mastomys natalensis* (Rodentia: Muridae) populations. *Acta Theriologica* **54:** 171–181.

Le Boulengé E, Legendre P, de le Court C, Le Boulengé -Nguyen P, Languy M. 1996. Microgeographic morphological differentiation in muskrats. *Journal of Mammalogy* **77:** 684–701.

Ledevin R, Millien V. 2013. Congruent morphological and genetic differentiation as a signature of range expansion in a fragmented landscape. *Ecology and Evolution* **3:** 4172–4182.

Ledevin R, Quéré J-P, Michaux JR, Renaud S. 2012. Can tooth differentiation help to understand species coexistence? The case of wood mice in China. *Journal of Zoological Systematics and Evolutionary Research* **50:** 315–327.

Macholán M, Mikula O, Vohralík V. 2008. Geographic phenetic variation of two eastern-Mediterranean non-commensal mouse species. *Mus macedonicus* and *M. cypriacus* (Rodentia: Muridae) based on traditional and geometric approaches to morphometrics. *Zoologischer Anzeiger* **247:** 67–80.

Markham BL, Barker JL. 1986. Landsat MSS and TM post-calibration dynamic rangers, exoatmospheric reflectance and at-satellite temperatures. *EOSAT Landsat Technology Notes* **1:** 3–8.

Martínez JJ, Di Cola V. 2011. Geographic distribution and phenetic skull variation in two close species of *Graomys* (Rodentia, Cricetidae, Sigmodontinae). *Zoologischer Anzeiger* **250:** 175–194.

Medan D, Torretta JP, Hodara K, de la Fuente EB, Montaldo NH. 2011. Effects of agriculture expansion and intensification on the vertebrate and invertebrate diversity in the Pampas of Argentina. *Biodiversity and Conservation* **20:** 3077–3100.

Millien V. 2004. Relative effects of climate change, isolation and competition on body size evolution in the Japanese field mouse *Apodemus argenteus*. *Journal of Biogeography* **31:** 1267–1276.

Millien V, Lyons SK, Olson L, Smith FA, Wilson AB, Yom-Tov Y. 2006. Ecotypic variation in the context of global climate change, revisiting the rules. *Ecology Letters* **9:** 853–869.

Millien-Parra V, Loreau M. 2000. Community composition and size structure of murid rodents in relation to the biogeography of the Japanese archipelago. *Ecography* **23:** 413–423.

Mills JM, Ellis BA, McKee KT, Maiztegui JI, Childs JE. 1992. Reproductive characteristics of rodent assemblages in cultivated regions of central Argentina. *Journal of Mammalogy* **73:** 515–526.

Monteiro LR, Duarte LC, dos Reis SF. 2003. Environmental correlates of geographical variation in skull and mandible shape of the punare rat *Trichomys apereoides*. *Journal of Zoology* **261:** 47–57.

Mora MS, Mapelli FJ, Gaggiotti OE, Kittlein MJ, Lessa EP. 2010. Dispersal and population structure at different spatial scales in the subterranean rodent *Ctenomys australis*. *BMC Genetics* **11:** 9.

Myneni RB, Hall FG, Sellers PJ, Marshak AL. 1995. The interpretation of spectral vegetation indexes. *IEEE Transactions Geoscience and Remote Sensing* **33:** 481–486.

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2011. *vegan: Community ecology package*. R package version 2.1-17. Available at: http://cran.r-project.org/web/packages/vegan/index.html [Accessed July 2012].

Parra V, Loreau M, Jaeger J-J. 1999. Incisor size and community structure in rodents: two tests of the role of competition. *Acta Oecologica* **20:** 93–101.

Peres-Neto PR, Legendre P, Dray S, Borcard D. 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology* **87:** 2614–2625.

Pergams ORW, Lacy RC. 2007. Rapid morphological and genetic change in Chicago-area *Peromyscus*. *Molecular Ecology* **17:** 450–463.

Pergams ORW, Lawler JJ. 2009. Recent and widespread rapid morphological change in rodents. *PLoS One* **4:** e6452.

Piras P, Marcolini F, Raia P, Curcio M, Kotsakis T. 2010. Ecophenotypic variation and phylogenetic inheritance in first lower molar shape of extant Italian populations of *Microtus* (*Terricola*) *savii* (Rodentia). *Biological Journal of the Linnean Society* **99:** 632–647.

Polly PD. 2003. Paleophylogeography: the tempo of geographic differentiation in marmots (*Marmota*). *Journal of Mammalogy* **84:** 369–384.

Polop JJ. 1996. Análisis de la respuesta adaptativa del género *Calomys*. Unpublished D. Phil. Thesis, Universidad Nacional de Río Cuarto, Río Cuarto.

Polop JJ, Sabattini MS. 1993. Rodent abundance and distribution in habitats of agrocenosis in Argentina. *Studies in Neotropical Fauna and Environment* **28:** 39–46.

R Development Core Team. 2012. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Renaud S, Michaux JR. 2003. Adaptive latitudinal trends in the mandible shape of *Apodemus* wood mice. *Journal of Biogeography* **30:** 1617–1628.

Renaud S, Millien V. 2001. Intra- and interspecific morphological variation in the field mouse species *Apodemus argenteus* and *A. speciosus* in the Japanese archipelago: the role of insular isolation and biogeographic gradients. *Biological Journal of the Linnean Society* **74:** 557–669.

Riesler LJ, Apodaca JJ. 2007. Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Systematic Biology* **56:** 924–942.

Rogic A, Tessier N, Legendre P, Lapointe F-J, Millien V. 2013. Genetic structure of the white-footed mouse in the context of the emergence of Lyme disease in southern Québec. *Ecology and Evolution* **3:** 2075–2088.

Rohlf FJ, Corti M. 2000. Use of two-block partial least-squares to study covariation in shape. *Systematic Biology* **49:** 740–753.

Rohlf FJ, Slice D. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39:** 40–59.

Schott JR, Volchok WJ. 1985. Thematic mapper thermal infrared calibration. *Photogrammetric Engineering and Remote Sensing* **51:** 1351–1357.

Simone I. 2010. Variaciones en la abundancia de *Calomys musculinus* (Rodentia: Cricetidae) y su relación con variables ambientales en bordes de cultivo. Unpublished D. Phil. Thesis, Universidad Nacional de Río Cuarto, Argentina.

Simone I, Cagnacci F, Provensal C, Polop J. 2010. Environmental determinants of the small mammal assemblage in an agroecosystem of central Argentina: the role of *Calomys musculinus*. *Mammalian Biology* **75:** 496–509.

Soriano A. 1991. Río de la Plata grasslands. In: Coupland RT, ed. *Natural grasslands, introduction and western hemisphere*. Amsterdam: Elsevier, 367–407.

Steinmann AR, Priotto JW, Castillo EA, Polop JJ. 2005. Size and overlap of home range in *Calomys musculinus* (Muridae: Sigmodontinae). *Acta Theriologica* **50:** 197–206.

Steinmann AR, Priotto JW, Polop JJ. 2009. Territorial behaviour in corn mice, *Calomys musculinus* (Muridae, Sigmodontinae) with regard to mating system. *Journal of Ethology* **27:** 51–58.

Suárez OV. 1994. Diet and habitat selection of *Oxymycterus rutilans* (Rodentia, Cricetidae). *Mammalia* **58:** 225–234.

Suárez OV, Bonaventura SM. 2001. Habitat use and diet in sympatric species of rodents of the low Parana delta, Argentina. *Mammalia* **65:** 167–176.

Suárez OV, Cueto GR, Kravetz FO. 1998. Sexual dimorphism according to age in *Oxymycterus rutilans* (Rodentia, Muridae). *Mastozoologia Neotropical* **5:** 129–136.

Teplitsky C, Millien V. 2014. Climate warming and Bergmann's rule through time: is there any evidence? *Evolutionary Applications* **7:** 156–168.

Tolliver DK, Choate JR, Kaufman DW, Kaufman GA. 1987. Microgeographic variation of morphometric and electrophoretic characters in *Peromyscus leucopus*. *American Midland Naturalist* **117:** 420–427.

Trimarchi LI. 2012. Patrones filogeográficos de *Akodon azarae* en ambientes fragmentados del centro-este argentino. Unpublished Undergraduate Thesis, Universidad Nacional de Córdoba, Argentina.

Tucker CJ, Fung IY, Keeling CD, Gammon RH. 1986. Relationship between atmospheric CO_2 variations and a satellite-derived vegetation index. *Nature* **319:** 195–199.

Wukelic GE, Gibbons DE, Martucci LM, Foote HP. 1989. Radiometric calibration of Landsat Thematic Mapper thermal band. *Remote Sensing of Environment* **28:** 339–347.

Yezerinac SM, Lougheed SC, Handford P. 1992. Measurement error and morphometric studies: statistical power and observer experience. *Systematic Biology* **41:** 471–482.

Yom-Tov Y. 1991. Character displacement in the psammophile Gerbillidae of Israel. *Oikos* **60:** 173–179.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Landmarks digitized in skull and mandible for three species of sigmodontine rodents from Central Argentina. Skull: 1 = rostralmost point of upper incisors next to the midline; 2 = lateralmost point of incisive alveolus, 3 = rostral end of foramen incisive; 4 = caudal end of foramen incisive; 5 = rostralmost point of molar row, 6 = caudalmost point of molar row; 7 = caudalmost point of the suture between palatine bones and the rostral border of mesopterygoid fossa; 8 = rostralmost point of the Eustachian tube; 9 = suture between basisphenoid and basioccipital where it contacts the tympanic bulla; 10 = rostral end of the occipital foramen in the midline; 11 = caudal end of the occipital foramen in the midline; 12 = caudalmost point of occipital condyle; 13 = caudalmost point of occipital–tympanic bulla junction; 14 = caudal end of the external opening of the bony auditory canal; 15 = anterior inferior tympanic bulla; 16 = lateralmost point between palatal and pterygoid. Mandible (*C. musculinus* and *A. azarae*): 1 = antero-dorsal border of incisor alveolus; 2 = anterior edge of maxillary toothrow; 3 = anterior edge of third lower molar; 4 = tip of the coronoid process; 5 = most extreme point of the curvature between coronoid and condylar processes; 6 = rostralmost point of articular surface of condyle; 7 = caudalmost point of articular surface of condyle; 8 = anterior-most point of the curve between the condylar and angular processes; 9 = tip of angular process; 10 = dorsal-most point on the ventral border of the horizontal ramus; 11 = posterior extremity of mandibular symphysis. Mandible (*O. rufus*): 1 = antero-dorsal border of incisor alveolus; 2 = anterior edge of maxillary toothrow; 3 = anterior edge of third lower molar; 4 = tip of the coronoid process; 5 = posterior edge of coronoid process; 6 = anterior edge of condylar process; 7 = rostralmost point of articular surface of condyle; 8 = caudalmost point of articular surface of condyle; 9 = anterior-most point of the curve between the condylar and angular processes; 10 = tip of angular process; 11 = antero-ventral border of incisor alveolus.

Figure S2. Levels of variability of skull and mandible shape as described by the two first principal components of independent analysis of intraspecific shape variation. The box plots show the median, and the 25 and 75 percentiles as a box, whereas the whiskers represent minimum and maximum values. Cm, *C. musculinus*; Aa, *A. azarae*; Or, *O. rufus*.

Figure S3. Skull shape differentiation using individual scores of the first two principal components. The utilization of different symbols helps to visualize the skull phenotypic relationships among localities (see Fig. 1). The shape changes associated with the first principal components are depicted; these were exaggerated twofold to improve the visualization of shape differences.

Figure S4. Mandible shape differentiation using individual scores of the first two principal components. The utilization of different symbols helps to visualize the mandible phenotypic relationships among localities (see Fig. 1). The shape changes associated with the first principal components are depicted; these were exaggerated twofold to improve the visualization of shape differences.

Table S1. Pearson correlation coefficients for climatic and environmental variables per trap-line. Below diagonal Pearson correlation coefficients and above diagonal P values. BH, border height; BW, border width; RSW, roadside width; HI1–11, heterogeneity indices; Temp, temperature; NDVI, normalized difference vegetation index; Rainf, rainfall.

Table S2. Summary of number of species per trap-line and mean centroid size values of skulls and mandibles for *C. musculinus* (Cm), *A. azarae* (Aa) and *O. rufus* (Or) used in geometric morphometrics. Sample size per species and structure is shown between parentheses. Trap-lines as in Figure 1. *Akodon dolores* (Ad), *Calomys laucha* (Cl), *Calomys venustus* (Cv), *Graomys griseoflavus* (Gg), *Necromys lasiurus* (Nl), *Oligoryzomys flavescens* (Of).

Table S3. Correlations between body length, body mass and log centroid size of skull and mandible for the three species. Cm, *Calomys musculinus*; Aa, *Akodon azarae*; Or, *Oxymycterus rufus*. Log cs, logarithm of centroid size.

Table S4. Adjusted spatial variation of skull size and shape by Moran eigenvector map (MEM). Only eigenfunctions measuring positive autocorrelation were considered. Cm, *Calomys musculinus*; Aa, *Akodon azarae*; Or, *Oxymycterus rufus*.

Table S5. Adjusted spatial variation of jaw size and shape by Moran eigenvector map (MEM). Only eigenfunctions measuring positive autocorrelation were considered. Cm, *Calomys musculinus*; Aa, *Akodon azarae*; Or, *Oxymycterus rufus*.

Table S6. Multiple regression of skull and mandible centroid sizes onto environmental and climatic variables. R^2 using mean trap-lines and individual scores are provided. Regression coefficients for each variable are depicted when the regression model was significant.

Table S7. Coefficients of the multivariate multiple regressions of skull and mandible shapes using individual scores onto 13 environmental and climatic variables for the three species studied. Principal components were utilized as shape variables due to their uncorrelated nature. 'P model' correspond to the full multivariate multiple regression. '%var' indicates the percentage of variation accounted for by the first two principal components considered. The amount of explained variance by the independent variables is depicted (R^2).

Table S8. Two blocks-partial least squares coefficients and percentage of covariation (Covar%) between skull and mandible mean shape using individual scores and 13 environmental and climatic variables for the three species studied. Main coefficients related to the first two partial least squares axes are in bold.