ORIGINAL ARTICLE

Geological habitat template overrides late Quaternary climate change as a determinant of range dynamics and phylogeography in some habitat-specialist water beetles

Pedro Abellán[1]*, Paula Arribas[2] and Jens-Christian Svenning[1]

[1]*Ecoinformatics and Biodiversity Group, Department of Bioscience, Aarhus University, Ny Munkegade 114, DK-8000 Aarhus C, Denmark,* [2]*Departamento de Ecología e Hidrología, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain*

ABSTRACT

Aim We investigated the roles of lithology and climate in constraining the ranges of four co-distributed species of Iberian saline-habitat specialist water beetles (*Ochthebius glaber*, *Ochthebius notabilis*, *Enochrus falcarius* and *Nebrioporus baeticus*) across the late Quaternary and in shaping their geographical genetic structure. The aim was to improve our understanding of the effects of past climate changes on the biota of arid Mediterranean environments and of the relative importance of history and landscape on phylogeographical patterns.

Location Iberian Peninsula, Mediterranean.

Methods We combined species distribution modelling (SDM) and comparative phylogeography. We used a multi-model inference and model-averaging approach both for assessment of range determinants (climate and lithology) and for provision of spatially explicit estimates of the species current and Last Glacial Maximum (LGM) potential ranges. Potential LGM distributions were then contrasted with the phylogeographical and population expansion patterns as assessed using mitochondrial DNA sequence data. We also evaluated the relative importance of geographical distance, habitat resistance and historical isolation for genetic structure in a causal modelling framework.

Results Lithology poses a strong constraint on the distribution of Iberian saline-habitat specialist water beetles, with a variable, but generally moderate, additional influence by climate. The degree to which potential LGM distributions were reduced and fragmented decreased with increasing importance of lithology. These SDM-based suitability predictions were mostly congruent with phylogeographical and population genetic patterns across the study species, with stronger geographical structure in the genetic diversity of the more temperature-sensitive species (*O. glaber* and *E. falcarius*). Furthermore, while historical isolation was the only factor explaining genetic structure in the more temperature-sensitive species, lithology-controlled landscape configuration also played an important role for those species with more lithology-determined ranges (*O. notabilis* and *N. baeticus*).

Main conclusions Our data show that lithology is an important constraint on the distribution and range dynamics of endemic Iberian saline-habitat water beetles, in interaction with climate and long-term climate change, and overrides the latter in importance for some species. Hence, geological landscape structure and long-term history may codetermine the overall range and the distribution of genetic lineages in endemic species with specialized edaphic requirements.

*Correspondence: Pedro Abellán,
Ecoinformatics and Biodiversity Group,
Department of Bioscience, Aarhus University,
Ny Munkegade 114, DK-8000 Aarhus C,
Denmark.
E-mail: pedro.abellan@biology.au.dk

INTRODUCTION

The dramatic climate changes of the Quaternary, particularly the Last Glacial Maximum (LGM) *c.* 21 ka (thousand years ago), have influenced the demographic history, genetic diversity, and distribution of many species (Hewitt, 2000). In Europe, research on this topic has focused on arctic–alpine and forest species (Schmitt, 2007). As a result, the role of Quaternary climatic fluctuations in other environments remains less well understood. This is in large part true for arid Mediterranean lowlands (but see Muñoz *et al.*, 2008; Pérez-Collazos *et al.*, 2009; Ortego *et al.*, 2009) and in particular for their inland saline aquatic ecosystems, which are globally restricted to arid lands and in Europe occur only in the Mediterranean region (Williams, 2002). While inland saline aquatic ecosystems house high numbers of endemic species (Millán *et al.*, 2011), little is known about which factors determine their ranges and genetic diversity patterns.

Although climate is often assumed to be the main range-limiting factor (Gaston, 2003; Pearson & Dawson, 2003), other abiotic factors can prevent species from occupying climatically suitable areas (Gaston, 2003). In inland saline aquatic systems, environmental constraints are imposed by both climatic and geological conditions (Williams, 2002; Millán *et al.*, 2011). In Europe, natural inland saline aquatic ecosystems are associated with specific lithological conditions (Millán *et al.*, 2011), notably calcareous and evaporitic outcrops. Hence, for species requiring such saline habitats, geological substrate is likely to be an important range constraint.

As a consequence of these climatic and geological constraints, inland saline environments in the western Mediterranean are highly fragmented. Hence, taxa inhabiting them may be particularly influenced by the landscape structure (Abellán *et al.*, 2007; Ortego *et al.*, 2009, 2010). Previous phylogeographical studies of saline-habitat specialist water beetles (Abellán *et al.*, 2007, 2009) have revealed contrasting patterns of genetic structure among species, perhaps reflecting differential impacts of landscape structure (distribution of habitat patches) and past climatic events. Landscape-genetic approaches have most often been used to examine how current spatial features influence contemporary levels of connectivity and divergence among populations (Storfer *et al.*, 2010). Applying similar methods in a historical framework provides insights into the population-level processes that underlie phylogeographical patterns and how they have changed over time (Chan *et al.*, 2011).

In this study we combine species distribution modelling (SDM; Elith & Leathwick, 2009) and comparative phylogeography for four co-distributed species of Iberian saline-habitat specialist water beetles to investigate the roles of lithology and climate in constraining their ranges across the late Quaternary and in shaping their genetic structure. Our specific hypotheses were threefold. (1) Lithology forms an important range constraint for Iberian saline-habitat water beetles. (2) Species for which lithology is the dominant present-day range constraint have not been affected by glacial–interglacial climate changes (i.e. they have had a relatively static range and population size during the late Quaternary); in contrast, species whose present-day ranges are greatly affected by current climate have experienced population and range expansion since the LGM. (3) In the former species, phylogeographical structure reflects mainly habitat connectivity; in contrast, in the latter species, with contracted, disjunct LGM ranges, phylogeographical structure mainly reflects these refugia.

MATERIALS AND METHODS

Study species

We focus on four species of water beetles of inland saline environments in the Iberian Peninsula: *Ochthebius glaber* Montes & Soler, *Ochthebius notabilis* Rosenhauer, *Nebrioporus baeticus* (Schaum) and *Enochrus falcarius* (Hebauer). All are restricted to saline and hypersaline waters and represent characteristic macro-invertebrates of this habitat in the Iberian Peninsula (Millán *et al.*, 2011). *Ochthebius glaber* is restricted to hypersaline streams in the south of the Iberian Peninsula. *Ochthebius notabilis* is found across the Iberian Peninsula and northern Africa (Jäch, 1992), inhabiting stagnant water bodies. *Nebrioporus baeticus* is distributed from the south of the Iberian Peninsula to the Pyrenees, occurring in saline streams. Finally, *Enochrus falcarius* has traditionally been viewed as a species widely distributed across the western Mediterranean. However, a recent study has revealed that this species as currently understood actually comprises a complex of distinct lineages, with small allopatric distributions (Arribas *et al.*, 2011). Here, we focused on the Iberian lineage of this species complex ('*E. falcarius* IP' *sensu* Arribas *et al.*, 2011; here *E. falcarius* for simplicity), restricted to saline streams in the southern Iberian Peninsula.

Species distribution modelling

We used SDM to investigate the relative role of current climate and lithology in constraining the distribution of the four

species, as well as to estimate their present and past (LGM) distributions as a basis for explaining geographical patterns in their genetic diversity.

Occurrence data and predictor variables

Occurrence data were compiled from previous studies (Abellán *et al.*, 2009; Arribas *et al.*, 2011) as well as from intensive recent sampling of inland saline habitats across the Iberian Peninsula (A. Millán *et al.*, University of Murcia, unpublished data). Occurrence localities for the various species represent most (if not all) known Iberian populations (see Fig. 1, and Table S1 in Appendix S1 in Supporting Information).

For the present-day SDM we initially considered the 19 bioclimatic variables from the WorldClim dataset at a spatial resolution of 2.5′ (http://www.worldclim.org/; Hijmans *et al.*, 2005). For the LGM (*c.* 21 ka) we used climate reconstructions of the same 19 bioclimatic variables based on two general atmospheric circulation models (GCM): the Community Climate System Model (CCSM; Collins *et al.*, 2006) and the Model for Interdisciplinary Research on Climate (MIROC v. 3.2; Hasumi & Emori, 2004) from the Paleoclimate Modelling Intercomparison Project Phase II (PMIP2) made available via WorldClim. To avoid multicollinearity problems, only a single variable from sets of highly correlated variables ($r > 0.9$) was used in the modelling, leaving a final set of 10 climatic predictor variables (Table 1). To account for geological range constraints, we additionally used lithology (dominant parent material) derived from the European Soil Database v. 2 (Van Liedekerke *et al.*, 2006) as a categorical predictor variable,
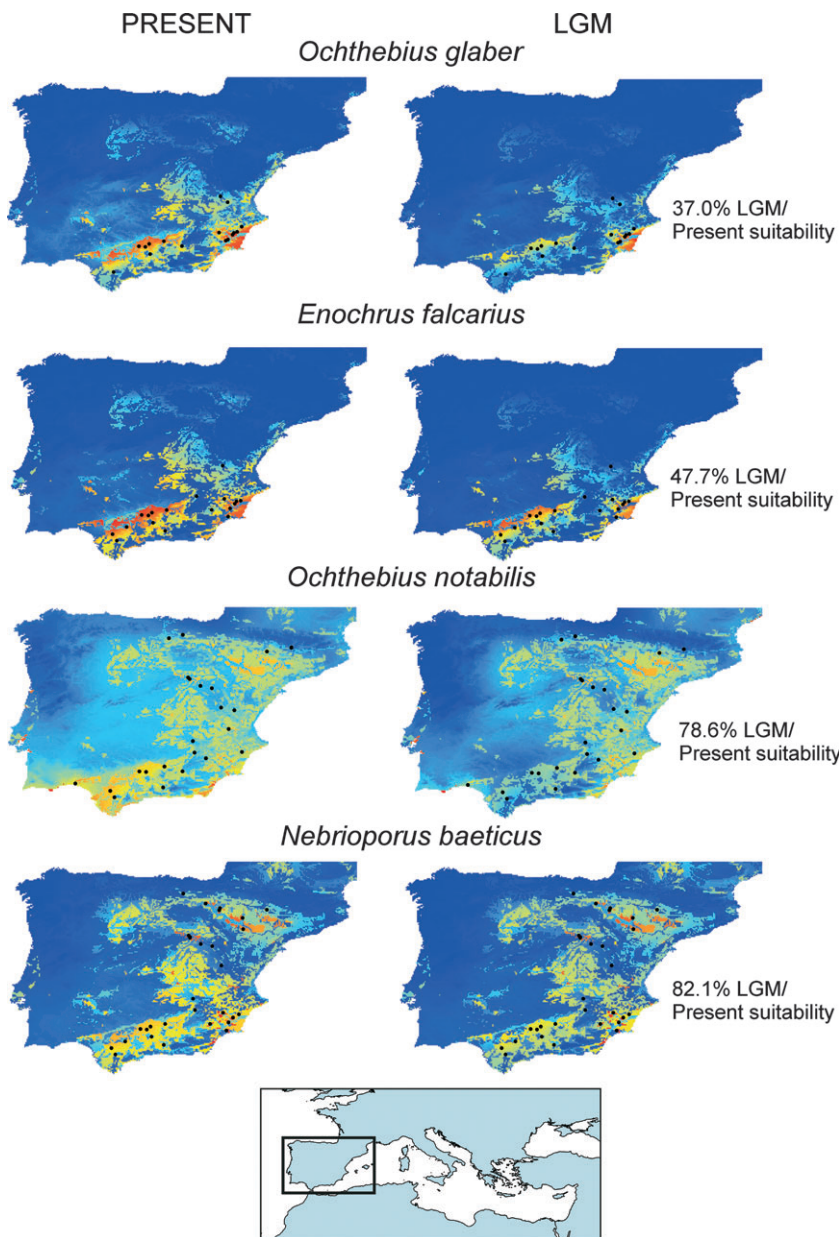
Figure 1 Species distribution modelling predictions for the four species of Iberian saline-habitat water beetles for the present (left) and the Last Glacial Maximum (LGM, right) obtained with Maxent. Warmer colours represent areas of higher habitat suitability. Black points represent present-day occurrence data used in the modelling. The percentage of the summed habitat suitability at the LGM relative to the present is also indicated. The inset shows the position of the study area in the Mediterranean Basin.

Table 1 Multi-model-average relative contributions (%) of environmental predictors in the Maxent distribution models for each of the four species of Iberian saline-habitat water beetles, assessed by averaging the influence of each variable in the confidence set of models, weighted by the Akaike weight.

Variable	Ochthebius glaber	Enochrus falcarius	Ochthebius notabilis	Nebrioporus baeticus
Lithology	49.96	59.89	69.81	88.68
Mean temperature of the driest quarter	32.22	22.78	0.00	5.96
Precipitation of the driest quarter	1.73	10.50	23.56	1.67
Annual precipitation	3.14	0.47	5.91	0.27
Annual mean temperature	7.45	1.75	0.00	0.03
Isothermality	3.13	0.63	0.00	0.60
Temperature annual range	1.44	0.13	0.00	1.12
Mean temperature of the wettest quarter	0.43	0.00	0.01	0.00
Minimum temperature of the coldest month	0.12	0.00	0.04	0.02
Maximum temperature of the warmest month	0.08	3.60	0.00	0.00
Precipitation seasonality	0.02	0.27	0.62	1.70

encompassing 43 categories of geological material in the Iberian Peninsula.

Modelling algorithm, model selection and multi-model inference and prediction

We modelled species distributions using the maximum-entropy machine-learning SDM algorithm Maxent v. 3.3.3e (Phillips & Dudík, 2008). Maxent is applicable to presence-only data and is considered to be among the most accurate of SDM algorithms (Elith *et al.*, 2006). To avoid basing our results solely on a single distribution modelling algorithm, we performed supplementary analyses using the Mahalanobis distance approach (MD; Farber & Kadmon, 2003). MD ranks potential sites by their Mahalanobis distance to a vector expressing the mean environmental conditions (the centroid) of all the species' records in the environmental space, and it requires neither absence data nor a definition of study extent.

We were interested in the relative performance of models with only climatic predictors compared with those also including lithology. Hence, two models were calibrated for each species with Maxent: one using the 10 selected climatic predictors, and the other one additionally including lithology as a categorical predictor. We then assessed the support for these sets of predictors using several approaches to evaluate model performance. The area under the receiver operating characteristic curve (AUC) was computed using Maxent's internal validation procedure in order to evaluate the predictive ability of each model (Phillips & Dudík, 2008). Although AUC is widely used to estimate the predictive accuracy in SDM, recent studies have highlighted limitations, especially with regard to its application to presence-only data (e.g. Lobo *et al.*, 2008). An additional and important issue concerning model performance in SDM is model complexity: Maxent may overfit species-predictor relationships, especially with small sample sizes, and overfitting will induce under-prediction when the distribution model is applied to new climatic scenarios (Warren & Seifert, 2011). In Maxent, model

complexity is constrained through regularization, which allows users to constrain over-parameterization by specifying a regularization multiplier parameter (β) that penalizes the addition of parameters (Phillips & Dudík, 2008). Warren & Seifert (2011) have shown the utility of information-theoretic approaches for setting regularization in Maxent, demonstrating that criteria such as AIC (Akaike's information criterion) may offer significant advantages. We computed various Maxent models by varying the regularization multiplier parameter (from 0.5 to 4) and allowing feature types (product, linear, quadratic, hinge and threshold; Phillips & Dudík, 2008). In all, we therefore obtained 72 distinct models (36 with only climatic predictors and 36 with both climatic variables and lithology; Table S2) for each species. We assessed the relative support for each model using sample-size-corrected AIC (AIC$_c$) (Burnham & Anderson, 1998), using ENMTools (Warren *et al.*, 2010). We then obtained a confidence set of models for each species: each model's AIC$_c$ was rescaled (ΔAIC) by subtracting the minimum AIC$_c$ value in the model set; that is, the best model has ΔAIC$_c = 0$ (Burnham & Anderson, 1998). Models with ΔAIC$_c > 10$ were discarded, as having essentially no support (Burnham & Anderson, 1998). We also excluded models with more parameters than data points (which violates the assumptions of AIC$_c$; Warren & Seifert, 2011). Akaike weights were then used to measure the probability that a given model was the best model for the observed data within the set of candidate models (Burnham & Anderson, 1998). We then used a multi-model inference and model-averaging approach both for assessment of range determinants and for providing spatially explicit estimates of the species current and LGM potential ranges, that is, areas with suitable environmental conditions. The multi-model-average relative contribution (%) of each environmental predictor in the Maxent distribution models for each species was assessed by averaging its influence across the confidence set of models, weighted by the Akaike weight. Potential-range predictions were likewise obtained by averaging predictions across the confidence set of models, weighted by the Akaike

weight; thereby, we reduced model bias and accounted for model uncertainty (Burnham & Anderson, 1998).

The supplementary Mahalanobis distance analyses were performed on the final model variable sets from the MAXENT analyses for each species (Table 1) using the Mahalanobis distance ARCVIEW extension (Jenness, 2003). Lithology was transformed into presence/absence of potentially saline substrates (marls and evaporitic rocks). The Mahalanobis distances were converted into probability values, rescaled to be in the range 0–1 according to the χ^2 distribution (Farber & Kadmon, 2003).

Model-averaged LGM predictions under CCSM and MIROC simulations were obtained as described above, with a subsequent averaging across the final CCSM and MIROC predictions. Because the geology is unlikely to have changed significantly since the LGM, we treated the lithology as constant.

To obtain binary predictions for the present and LGM to be used in the phylogeographical analyses, we converted the averaged, continuous MAXENT outputs into presence/absence predictions using maximum training sensitivity plus specificity thresholding, which has been found to perform well compared with other thresholding methods (Liu *et al.*, 2005).

Phylogeographical analyses

Genetic data

Mitochondrial DNA (mtDNA) sequences (3′ end of cytochrome *c* oxidase subunit I, *COI*) for the four species were obtained from previous studies (Abellán *et al.*, 2007, 2009; Arribas *et al.*, 2011; P. Arribas *et al.*, unpublished results). A total of 342 sequenced specimens of *O. glaber* (90), *E. falcarius* (72), *N. baeticus* (93) and *O. notabilis* (87) were used, covering their known geographical ranges with a mean of five individuals per locality (see Table S1; GenBank accession numbers for sequences of *E. falcarius* are indicated in Table S3).

Genetic diversity, population structure and haplotype relationships

Standard genetic diversity indices were estimated using DNASP v. 5 (Librado & Rozas, 2009). Genetic differentiation between populations with ≥ 4 sequenced individuals was quantified by pairwise Φ_{ST} and tested for significance by 10,000 permutations using ARLEQUIN v. 3.5 (Excoffier & Lischer, 2010).

Potential LGM distributions were compared with the phylogenetic relationships among mtDNA haplotypes. Networks of the mitochondrial *COI* haplotypes for *E. falcarius* were inferred using statistical parsimony with TCS v. 1.13 (Clement *et al.*, 2000), while networks for *O. glaber*, *O. notabilis* and *N. baeticus* were taken from Abellán *et al.* (2009).

Analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) was used to assess the partitioning of genetic variation among and within biogeographical regions as represented by the main Iberian river basins (see Fig. 2). AMOVAs were performed using ARLEQUIN, using 10,000 permutations. Individual collecting localities were treated as populations.

### Estimating variation in effective population size over time

We tested for post-glacial range expansions using two genetic approaches, for comparison with SDM-based estimates of range stasis or expansion.

The demographic history of every biogeographical region (with ≥ 10 individuals) for each species was inferred based on mismatch distributions of pairwise nucleotide differences (Rogers & Harpending, 1992) with 1000 bootstrap replications in ARLEQUIN. The goodness-of-fit between the observed and expected distributions of demographic models, including sudden expansion and spatial expansion, was calculated using sum of square deviations (SSD; Schneider & Excoffier, 1999). The raggedness index (r) of the observed mismatch distribution was also calculated for each of the populations, and its significance determined, as implemented in ARLEQUIN. Non-significant deviations of expected and observed mismatch distributions and low raggedness values indicate past population expansion (Rogers & Harpending, 1992). For expanding populations, tau (τ, calculated from the mismatch distribution; see Schneider & Excoffier, 1999) was used to estimate time since expansion (t) using the equation $\tau = 2\,\mu t$, where μ is the neutral mutation rate for the locus (Schneider & Excoffier, 1999). The confidence intervals for τ were calculated using parametric bootstrap (Schneider & Excoffier, 1999). Divergence rates for *COI* in Coleoptera range from 3.34 to 4.00% (Papadopoulou *et al.*, 2010; Ribera *et al.*, 2010). We used the average of these values (3.62% Myr^{-1}), and assumed a 1-year generation time.

Tajima's *D* (Tajima, 1989) and Fu's F_S (Fu, 1997) are sensitive to departures from mutation–drift equilibrium as a result of population size changes (expansions, bottlenecks) and selection, and were computed for the biogeographical regions using ARLEQUIN. Significant negative deviations of these indexes from zero are interpreted as evidence of either selective sweeps or population expansions (Fu, 1997).

Testing landscape and historical effects on genetic structure

The SDM estimates of potential ranges were also used to directly test alternative phylogeographical scenarios. The genetic structure in species of limited dispersal ability inhabiting patchy habitats might be expected simply to reflect geographical distance among habitat patches. Alternatively, landscape characteristics may influence gene flow between populations by affecting dispersal rates and the spatial arrangement among them. Such landscape effects may be represented by resistance surfaces that assign different resistance-to-movement values to different landscape features (Storfer *et al.*, 2010). If such landscape effects have been important, we would expect a positive relationship between genetic distance and landscape resistance, after controlling for geographical distance or history (see below). We estimated
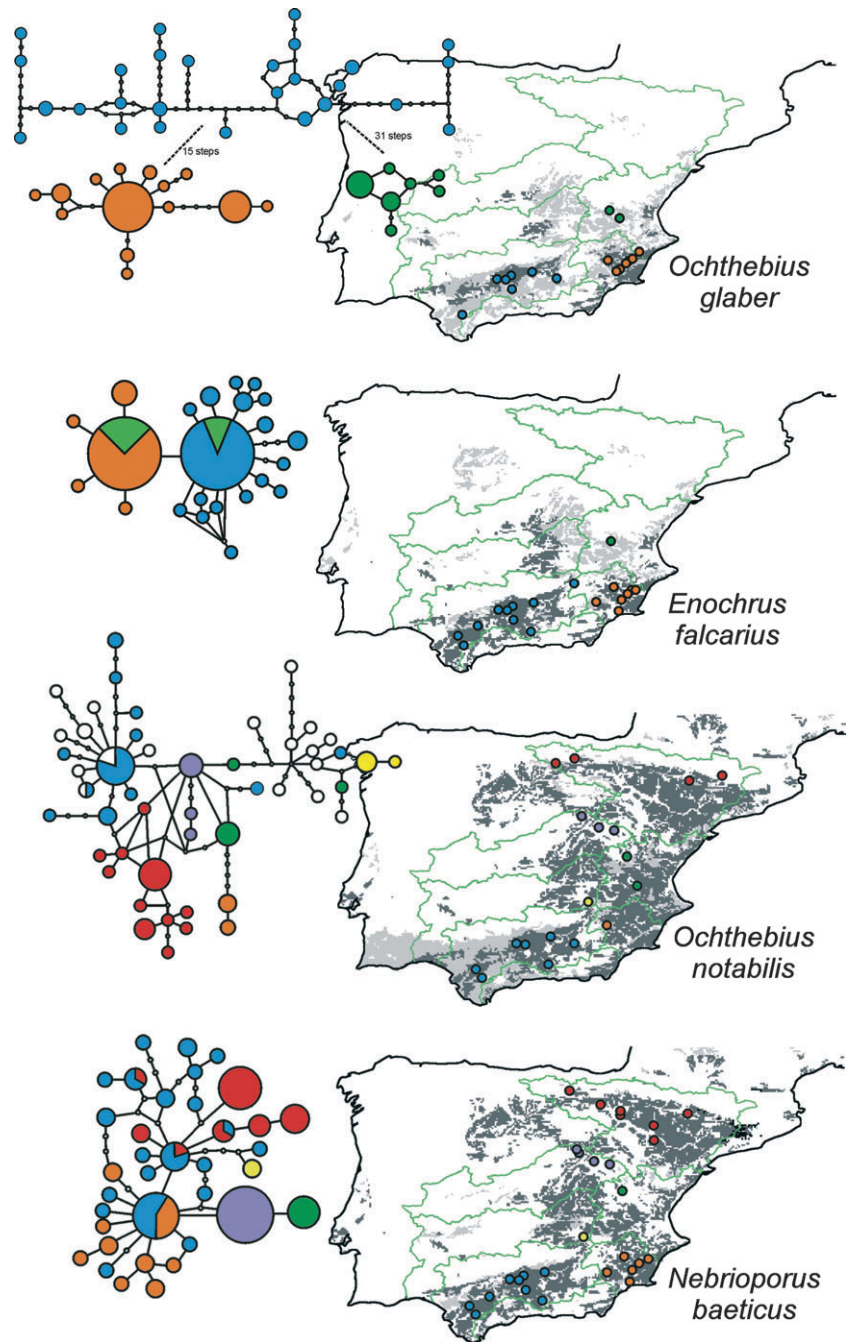
Figure 2 Comparison between present and Last Glacial Maximum (LGM) potential ranges and phylogenetic relationships among mtDNA haplotypes of the four species of Iberian saline-habitat water beetles (statistical parsimony networks). Shaded areas correspond to suitable areas: dark grey, stable areas (i.e. suitable during both time periods); light grey, present distributional areas predicted to have been unsuitable during the LGM; and black, currently unsuitable areas predicted to have been suitable during the LGM. The boundaries of the main river basins are indicated by green lines. Populations used in genetic analyses are shown in the maps with coloured circles (different colours represent the various main regions: blue, Guadalquivir; orange, Segura; green, Júcar; red, Ebro; yellow, Guadiana; purple, Tajo). In cytochrome *c* oxidase subunit I haplotype networks, solid lines connect haplotypes with a single step (missing intermediates are indicated by small open circles). Circle diameter is proportional to the number of individuals. White haplotypes in *Ochthebius notabilis* correspond to Moroccan populations.

landscape resistance by inverting the SDM estimates of habitat suitability (Chan *et al.*, 2011), accounting for changes across time by estimating resistance for both the LGM and the present. Matrices of resistance distances among population pairs were computed with the software CIRCUITSCAPE v. 2.2 (McRae & Shah, 2009), which uses circuit theory to evaluate the total landscape resistance between sampling sites based on multiple paths.

If historical isolation in biogeographical regions (main river basins) during the late Quaternary has played a major role in shaping genetic structure, we would expect strong genetic differentiation between the regions after controlling by

geographical distance or landscape characteristics. Historical geographical structure was represented by a matrix, where locality pairs were assigned a value of 1 if in the same region, and 0 otherwise.

We then evaluated the relative importance of geographical distance, habitat resistance and historical isolation for genetic structure using Mantel tests and partial Mantel tests in a causal modelling framework (Cushman *et al.*, 2006). We identified the diagnostic expectations for each hypothesis of causal relationships (Table 2). Thus, the model with best support should exhibit not only the highest simple correlation with genetic distance, but also a significant, positive partial corre-

Table 2 Causal modelling framework for phylogenetic inference for Iberian saline-habitat water beetles based on genetic diversity. Diagnostic expectations (partial Mantel test and expected significance value) for each hypothesis: D (Euclidean distance), H (habitat resistance), R (historical regions, long-term isolation in main river basins), G (genetic distance). NS, not significant; > 0, significant positive correlation ($P < 0.05$); NA, not applicable. A period separates the main matrices on the left from the covariate matrix on the right that is controlled for in the partial Mantel test. For example, DG.H is a partial Mantel test between geographical and genetic distance after controlling for habitat resistance.

| | Models and diagnostic expectations | | | | | | |
Test	Geographical distance	Habitat resistance	Historical regions	Distance and habitat	Distance and regions	Habitat and regions	Distance, habitat, regions
DG.H	> 0	NS	NA	> 0	> 0	NS	> 0
DG.R	> 0	NA	NS	> 0	> 0	NS	> 0
HG.D	NS	> 0	NA	> 0	NS	> 0	> 0
HG.R	NA	> 0	NS	> 0	NS	> 0	> 0
RG.H	NA	NS	> 0	NS	> 0	> 0	> 0
RG.D	NS	NA	> 0	NS	> 0	> 0	> 0

lation with genetic distance after controlling for each competing factor. Mantel tests and partial Mantel tests were computed in the R software v. 2.13 (R Development Core Team, 2011) using the 'vegan' package (Oksanen *et al.*, 2007), with significance assessed using 10,000 permutations.

RESULTS

Species distribution models

The MAXENT models for all species provided strong support for the joint importance of climate and lithology (Table 3). For *E. falcarius*, *O. notabilis* and *N. baeticus*, confidence sets comprised only models that included both climate and lithology, because all models with only climatic predictors had essentially no support ($\Delta AIC_c > 10$; Table S2). For *O. glaber*, two strictly climatic models were included among the 12 models in the confidence set, but only with 0.3–3.2% support (Table S2). For those models with the highest support for each species, the probability that they were the best model for the observed data according to Akaike weights was always lower than 40%. In all cases, these most probable models included both climate and lithology (see Table S2). Models including lithology had significantly higher training AUC values than those including only climatic variables, as assessed

by *t*-tests (*O. glaber*, $t = 6.92$, $P < 0.001$; *E. falcarius*, $t = 11.43$, $P < 0.001$; *O. notabilis*, $t = 9.08$, $P < 0.001$; *N. baeticus*, $t = 10.16$, $P < 0.001$; see also Table 3).

In all species, multi-model inference identified lithology as the most important range predictor, with a high relative contribution (50–89%), with climate also providing an important contribution except in *N. baeticus* (Table 1). Among climatic variables, mean temperature of the driest quarter had the highest relative contribution in *O. glaber*, *E. falcarius* and *N. baeticus* (although accounting for only 6% of relative contribution in this last species), while precipitation of the driest quarter was the most important climatic variable in *O. notabilis* (Table 1). Hence, temperature plays a major role only for *O. glaber* and *E. falcarius*.

In general, the multi-model average MAXENT predictions matched reported species distributions (Figs 1 & 2). The models also predicted suitable conditions outside their reported distribution, especially in central Spain for *O. glaber* and *E. falcarius*, and to the north-west of their current distribution for *O. notabilis* and *N. baeticus*.

Projecting these models to the LGM, the estimated distributions did not differ dramatically from those of the present day, although the temperature-sensitive *O. glaber* and *E. falcarius* displayed markedly lower overall habitat suitability (Figs 1 & 2). Suitable areas were more fragmented and

Species	Model set	AUC	ΣW (%)
Ochthebius glaber	Climate	0.924 (0.885–0.974)	3.67
	Climate + lithology	0.955 (0.920–0.982)	96.33
Enochrus falcarius	Climate	0.920 (0.886–0.981)	~ 0
	Climate + lithology	0.970 (0.954–0.990)	~ 100
Ochthebius notabilis	Climate	0.749 (0.635–0.928)	~ 0
	Climate + lithology	0.879 (0.820–0.948)	~ 100
Nebrioporus baeticus	Climate	0.796 (0.719–0.923)	~ 0
	Climate + lithology	0.904 (0.873–0.954)	~ 100

Table 3 Comparisons of the predictive ability and model support among species distribution models for the four species of Iberian saline-habitat water beetles, based on two different sets of predictors (climate and climate plus lithology). AUC, area under the receiver operating characteristic curve for training data; ΣW, summed Akaike weights.

discontinuous at the LGM for all species, but especially for two temperature-sensitive species, notably in terms of reduced continuity between their current main areas of distribution.

The Mahalanobis distance modelling provided predictions that were consistent with the MAXENT results for both the present and the LGM (Fig. S1), with potential ranges for *O. glaber* and *E. falcarius* restricted mainly to southern Iberia and more fragmented than those for *O. notabilis* and *N. baeticus*, especially at the LGM.

Phylogeographical structure and demographic changes

Genetic diversity was higher in the Guadalquivir basin (southern Iberia) than in the other regions for all species

(Table 4). AMOVAs detected significant genetic structuring among populations grouped by the main river basins of the Iberian Peninsula in the four species (Table 5). The proportion of overall variance accounted for by differences among regions differed markedly among species, being especially high for temperature-sensitive *O. glaber* and *E. falcarius*. Hence, these species had regional groups that were genetically relatively different from each other and internally more homogeneous than did the other species. The least climatically sensitive species, *N. baeticus*, provided the strongest contrast, with genetic differences within populations accounting for the highest proportion of variance. Correspondingly, haplotype networks in *O. glaber* and *E. falcarius* showed clear geographical structure with mostly no shared haplotypes among the main river basins; haplotype networks in *O. notabilis* and

Table 4 Genetic diversity, demographic statistics and mismatch distribution results for the four species of Iberian saline-habitat water beetles in the various regions. n, sample size; π, nucleotide diversity; h, number of haplotypes; H, haplotype diversity; D, Tajima's D-test; and F_S, Fu's F_S test (bold: P-value < 0.05). P(SDD), P-value for the sum of the squared deviations under the hypothesis of spatial expansion; P(Rag), P-value for the raggedness index (regions with sample sizes < 10 sequences are not shown). Tau (τ) was converted to thousand years ago (T, in ka) as an estimate of the time of expansion. CI, confidence interval. Main Iberian river basins: GUA, Guadalquivir; SEG, Segura; JUC, Júcar; EBR, Ebro; GUD, Guadiana; TAJ, Tajo.

| Region | n | π | h | H | F_S | D | Spatial expansion model | | | |
							P(SDD)	P(Rag)	τ (95% CI)	T (ka)
Ochthebius glaber										
GUA	42	0.014	27	0.970	**−14.094**	−1.014	0.596	0.896	6.8 (5.1–9.8)	117 (87–168)
SEG	32	0.004	14	0.791	**−4.202**	−0.914	0.765	0.852	2.9 (1–6.1)	50 (18–104)
JUC	16	0.002	7	0.775	**−3.228**	−1.025	0.521	0.429	1.4 (0.6–2.3)	24 (10–39)
Enochrus falcarius										
GUA	42	0.003	18	0.664	**−3.877**	−1.625	0.67	0.601	2.6 (0.3–5.1)	45 (6–88)
SEG	24	0.001	5	0.529	**−1.350**	−0.916	0.045	0.283	0.6 (0.3–1.4)	10 (5–24)
JUC	6	0.001	2	0.533	0.625	0.851	–	–	–	–
Ochthebius notabilis										
GUA	23	0.005	13	0.874	**−5.751**	−1.413	0.79	0.881	3.6 (1.3–5.3)	62 (23–92)
SEG	5	0.001	2	0.600	0.626	1.225	–	–	–	–
JUC	7	0.002	3	0.524	0.541	−0.876	–	–	–	–
EBR	21	0.004	9	0.757	**−3.868**	−0.684	0.600	0.729	2.7 (0.8–4.2)	46 (14–72)
GUD	5	0.001	2	0.400	0.090	−817	–	–	–	–
TAJ	7	0.004	2	0.476	2.508	0.755	–	–	–	–
Nebrioporus baeticus										
GUA	42	0.005	20	0.950	**−14.530**	−1.571	0.645	0.826	2.9 (1.9–4.7)	50 (33–81)
SEG	16	0.003	9	0.917	**−4.130**	−0.931	0.402	0.323	2.2 (1–3.2)	39 (17–55)
JUC	6	0.000	1	0.000	–	–	–	–	–	–
EBR	23	0.004	7	0.798	0.162	0.180	0.811	0.843	3.6 (1.2–6.3)	62 (21–109)
GUD	2	0.000	1	0.000	–	–	–	–	–	–
TAJ	13	0.000	1	0.000	–	–	–	–	–	–

Table 5 Results of the analyses of molecular variance with individuals of the four species of Iberian saline-habitat water beetles grouped by main regions (river basins) and populations. The percentage of total variance and P-value (in parentheses) are shown.

	Ochthebius glaber	*Enochrus falcarius*	*Ochthebius notabilis*	*Nebrioporus baeticus*
Among regions	83.26 (< 0.001)	50.65 (< 0.001)	42.11 (< 0.001)	36.83 (< 0.001)
Among populations within regions	6.10 (< 0.001)	7.42 (0.005)	28.78 (< 0.001)	16.44 (< 0.001)
Within populations	10.63 (< 0.001)	41.93 (< 0.001)	29.15 (< 0.001)	46.74 (< 0.001)

N. baeticus showed more complex patterns, with shared haplotypes among some regions and high levels of homoplasy (Fig. 2).

Negative and significant values of Fu's F_S were obtained for nearly all the regions (excluding those with small sample sizes) and species (Table 4). These values represented significant departures from those expected under a null model of demographic equilibrium and genetic drift. With respect to Tajima's *D*, although most regions showed negative values, these were significant only in Guadalquivir basin for *E. falcarius* and *N. baeticus*. The mismatch distributions were mostly not significantly different from those expected under either a spatial expansion or a sudden demographic expansion (Table 4; see also Table S4 and Fig. S2). The estimated times at which these expansions occurred for the different regions in each species were variable, but all were in the late Quaternary (Table 4).

Landscape and historical effects on genetic structure

In all species, genetic distance (pairwise Φ_{ST}, Table S5) had significant correlations with all tested spatial factors (Euclidean distance, habitat resistance at both the present and LGM, and main river basin regions; Table 6). In the temperature-sensitive *O. glaber* and *E. falcarius*, however, genetic distance was most strongly correlated with river basins, corresponding to long-term isolation in these regions (see Fig. 2). Furthermore, in these species the other spatial factors had no importance after controlling for the river basin effect. In contrast, present habitat connectivity (see Fig. S3) had the strongest relationship with genetic distance in *O. notabilis* and *N. baeticus* (although river basin regions had similar correlations). Interestingly, in these species the correlation between genetic distance and habitat resistance was markedly stronger for present than for LGM habitat resistance. Most importantly,

it was only in these species that habitat resistance was significantly correlated with genetic distance after controlling for both geographical distance and the river basin region effect. Nevertheless, *O. notabilis* and *N. baeticus* also exhibited significant river basin region effects after controlling for the other factors.

According to the causal modelling framework (Table 2), the hypothesis of historical isolation in river basin regions is the only fully supported one for *O. glaber* and *E. falcarius*, while in *O. notabilis* and *N. baeticus* the hypothesis of isolation by both habitat resistance and main regions is the only fully supported one.

DISCUSSION

By integrating species distribution modelling with phylogeography, we can derive a more refined image of the driving forces that have led to the distributions of extant taxa and their genetic diversity (Kozak *et al.*, 2008; Svenning *et al.*, 2011). Our data show that lithology poses an important constraint on the distribution and range dynamics of Iberian saline-habitat water beetles, with climate playing a variable, but more moderate, role. We furthermore find that long-term history (linked to climate history) and geological habitat template interact in shaping the geographical genetic structure, with the former being more important for the species with more temperature-sensitive ranges, and habitat template being more important for species with more lithology-controlled ranges.

Predicted distributions, range dynamics and phylogeography

Species distribution models have become a common tool in studies of biogeography, conservation biology, ecology, evolutionary biology and palaeobiology (Elith & Leathwick,

Table 6 Mantel and partial Mantel correlations (*r*) between spatial and genetic pairwise distances of the four species of Iberian saline-habitat water beetles among localities (bold: *P*-value < 0.05). D, Euclidean distance; H_P, habitat resistance at the present; H_{LGM}, habitat resistance at the Last Glacial Maximum; R, regions (historical isolation in main river basins); G, genetic distance.

Test	*Ochthebius glaber*		*Enochrus falcarius*		*Ochthebius notabilis*		*Nebrioporus baeticus*	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value
DG	**0.704**	**0.000**	**0.518**	**0.002**	**0.391**	**0.002**	**0.294**	**0.001**
H_PG	**0.664**	**0.000**	**0.525**	**0.002**	**0.542**	**0.000**	**0.386**	**0.004**
H_{LGM}G	**0.621**	**0.000**	**0.524**	**0.003**	**0.387**	**0.010**	**0.272**	**0.022**
RG	**0.836**	**0.000**	**0.802**	**0.000**	**0.531**	**0.000**	**0.351**	**0.000**
DG.H_P	**0.317**	**0.002**	0.107	0.156	−0.094	0.217	0.124	0.061
DG.H_{LGM}	**0.489**	**0.000**	0.080	0.221	0.129	0.140	0.116	0.075
DG.R	0.097	0.141	−0.071	0.257	0.135	0.123	0.046	0.290
H_PG.D	0.054	0.359	0.147	0.114	**0.417**	**0.010**	**0.288**	**0.028**
H_{LGM}G.D	−0.270	**0.007**	0.123	0.157	0.112	0.289	0.009	0.466
H_PG.R	0.096	0.213	−0.171	0.120	**0.451**	**0.006**	**0.222**	**0.034**
H_{LGM}G.R	0.016	0.424	−0.125	0.175	0.291	0.057	0.057	0.343
RG.H_P	**0.682**	**0.000**	**0.723**	**0.000**	**0.435**	**0.003**	**0.144**	**0.009**
RG.H_{LGM}	**0.714**	**0.000**	**0.719**	**0.001**	**0.476**	**0.002**	**0.238**	**0.000**
RG.D	**0.638**	**0.000**	**0.718**	**0.000**	**0.409**	**0.005**	**0.207**	**0.001**

2009; Svenning *et al.*, 2011). Improving model selection, model evaluation and predictor contribution assessment are central challenges in the development of SDM (Elith & Leathwick, 2009). Here, we explore some of these issues: models for prediction need to balance fit to the training data against generality that enables reliable prediction to new cases (Elith & Leathwick, 2009). Although model complexity is not usually addressed, it can influence the ability of the model to assess the relative importance of variables in constraining species distributions, to infer habitat quality and its transferability to other time periods (Warren & Seifert, 2011). Our results show that Maxent models produced with default parameter settings were in many cases too complex, because they included more parameters than data points (Table S2), revealing that default regularization parameters do not always prevent Maxent from overfitting. The fact that predictions obtained with Mahalanobis distance were similar to those estimated using Maxent indicate that our SDM-based findings are not algorithm-dependent. Importantly, in contrast to Maxent, the Mahalanobis distance method relies exclusively on presences (i.e. it does not use pseudo-absences from 'background' environmental data) and is not extent-dependent. Hence, the congruence between the two modelling techniques suggests that past potential distributions estimated by the averaged Maxent models do not represent overfit or biased estimates.

The first aim of this study was to assess the relative role of climate and lithology for species distributions of saline-habitat water beetles. Our results show that non-climatic environmental factors such as lithology can be important not only at local scales (Thuiller *et al.*, 2004) but also at regional scales in habitat-specialist species (see also, for example, Gastón *et al.*, 2009): distributions of all four water beetles are importantly limited by lithology, with > 50% relative contribution in all species. In contrast, climatic factors influence distributions only moderately. Moreover, among climatic predictors, temperature plays a major role only for *O. glaber* and *E. falcarius*.

As a consequence of the limited influence of climate, inferred LGM distributions are not dramatically different from present-day distributions, although overall suitability is always reduced (Fig. 1). These findings suggest that species were able to persist throughout much of their current ranges during the late Pleistocene, with cold episodes (such as the LGM) leading mainly to diffuse population contraction. There is strong palynological (González-Sampériz *et al.*, 2010), biogeographical (Ribera & Blasco-Zumeta, 1998) and phylogeographical (Pérez-Collazos *et al.*, 2009) evidence supporting the persistence of suitable areas across the Iberian Peninsula for steppe and arid biota throughout the Pleistocene. Arid conditions were not interrupted during this period, at least in the Guadalquivir and Ebro basins (Plans, 1969), and phylogeographical studies that have focused on saline invertebrates suggest a scenario of persistence in multiple Iberian suitable areas (Gómez *et al.*, 2000; Ortego *et al.*, 2009; Pérez-Collazos *et al.*, 2009). For *O. glaber* and *E. falcarius*, climatic changes increased isolation among their main areas of occurrence (river

basins). In contrast, ranges of *O. notabilis* and *N. baeticus* are predicted to have remained more stable.

The SDM suitability predictions were mostly congruent with the genetic patterns. Both parsimony haplotype networks and AMOVAs revealed a strong geographical structure in the temperature-sensitive *O. glaber* and *E. falcarius*, in agreement with SDM-predicted historical isolation in their main areas of occurrence (Avise, 2000). The absence of shared haplotypes and the high genetic differentiation among regions coupled with the low genetic differentiation among populations within regions point to a scenario in which gene flow has been very restricted (Abellán *et al.*, 2007, 2009). Although AMOVAs also support that mtDNA haplotype variation is partitioned among the main Iberian regions in *O. notabilis* and *N. baeticus*, groups were less genetically homogeneous, and haplotype networks exhibited more complex geographical patterns, suggesting some gene flow among regions and the additional role of other factors (see below).

If ranges were more restricted in the past as a consequence of cold episodes, subsequent post-glacial range expansions must have been associated with demographic expansion (e.g. Lessa *et al.*, 2003). This is supported by the results of the mismatch distribution analysis. Although mismatch distribution analysis may have little power (Ramos-Onsins & Rozas, 2002), the indices Tajima's D and Fu's F_S also suggest population growth. The star-shaped pattern in the haplotype networks for *E. falcarius* and *O. glaber* in some regions also points to either rapid population expansion from a refugium or founder events (Avise, 2000), although at variable times during the late Quaternary, partially pre-LGM (Table 4). On the other hand, and despite the fact that paleoclimatic reconstructions point to a history of greater stability in habitat patches in *O. notabilis* and *N. baeticus*, we also found footprints of demographic expansion in most regions for these species, in agreement with the moderate overall reduction in suitability predicted for these species (Fig. 1). Nevertheless, time estimates again suggest pre-LGM demographic events. Hence, the latitudinal decrease of genetic diversity in all four species may reflect northward colonization events from southern Iberia and, ultimately, North Africa, with subsequent population growth (Pérez-Collazos *et al.*, 2009).

Effects of history and landscape on genetic structure

The causal modelling framework allowed further assessment of which factors determine genetic structure (Cushman *et al.*, 2006), disentangling effects of long-term history and geological landscape structure. In concordance with the phylogeographical and population genetic results, we found contrasting influences of the various spatial factors in the four species. Our findings show that historical river-basin structure plays an important role in explaining the genetic differentiation and population structure in all four species, in agreement with results for Iberian endemic fish (Gómez & Lunt, 2007) and other Iberian saline invertebrates (Gómez *et al.*, 2000; Ortego *et al.*, 2009, 2010). Nevertheless, while this historical

component was the only factor explaining genetic structure in the temperature-sensitive *O. glaber* and *E. falcarius* after removing the other effects of spatial factors, landscape configuration also played a crucial independent role in *O. notabilis* and *N. baeticus*.

The different phylogeographical patterns and the contrasting roles of historical and landscape factors in shaping genetic structure in *O. glaber* and *E. falcarius* versus *O. notabilis* and *N. baeticus* seem to be a consequence of their differential climatic sensitivity, perhaps coupled with differences in dispersal ability. Despite the fact that the four studied species are all able to fly, little is known regarding their dispersal. Dispersal ability for *O. glaber* seems to be limited, as suggested by its high degree of geographical genetic structure (Abellán *et al.*, 2007). In fact, differences in dispersal ability have already been suggested for explaining differences in phylogeography between *O. glaber* and *O. notabilis* (Abellán *et al.*, 2009), which occur in saline habitats with contrasting long-term stability (running and standing water bodies, respectively). Similarly, a recent study suggested reduced dispersal capability in *E. falcarius* in relation to its relatives inhabiting standing waters (Arribas *et al.*, 2011).

The impact of barriers to dispersal and gene flow is often inferred to be the primary cause of phylogeographical structure (Avise, 2000). All barriers ultimately represent regions of unsuitable ecological conditions for a species, reducing dispersal and affecting range limits (Gaston, 2003). According to the framework proposed by Pyron & Burbrink (2010), the differences in the phylogeographical patterns found between the two species pairs in relation to spatial factors could represent two contrasting modes of geographical isolation: physically mediated ('hard') and ecologically mediated ('soft') allopatric divergence. While in *O. glaber* and *E. falcarius* divergence would be a consequence of barriers representing discrete physiographic features – associated with unfavourable climatic conditions – limiting dispersal (e.g. Steeves *et al.*, 2005), divergence in *O. notabilis* and *N. baeticus* would be a consequence of their response to more continuously varying ecological conditions (e.g. Costa *et al.*, 2008). So, the configuration of the actual Iberian river basins represents a main factor for generating barriers and isolating populations in *O. glaber* and *E. falcarius*, with isolation increasing during Pleistocene cold episodes because of the limited dispersal capacity of these species coupled with their higher sensitivity to climatic conditions (see also Sánchez-Fernández *et al.*, 2011). In the putatively more vagile *O. notabilis* and *N. baeticus*, physiographical features and the geological landscape configuration would represent hierarchical spatial-temporal drivers (e.g. Koscinski *et al.*, 2009).

CONCLUSIONS

By exploring our data with an integrative approach, it was possible to reveal how climate and climatically driven historical fluctuations in population demography, and associated historical isolation, and geological habitat template interact to shape distributions and phylogeographical patterns in Iberian saline-habitat specialist water beetles. Our results emphasize the importance of multifaceted approaches combining ecological and genetic modelling to obtain improved insights into the determinants of range dynamics and phylogeography, as well as, more broadly, for assessing the effects of Quaternary climatic fluctuations on European biodiversity. Furthermore, from a conservation perspective, by identifying long-term barriers to gene flow, and the contrasting effects of climate and landscape on genetic diversity, it will be possible to model species responses to future environmental changes more realistically.

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SUPPORTING INFORMATION

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

Appendix S1 Additional data and results (Tables S1–S5 and Figs S1–S3).

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BIOSKETCHES

Pedro Abellán is a post-doctoral researcher in the Department of Bioscience, Aarhus University, Denmark. His research is currently focused on the biogeography and conservation of water beetles and the phylogeographical patterns of western Mediterranean biota.

Jens-Christian Svenning is a professor in the Department of Bioscience, Aarhus University, Denmark. His main research interests include ecoinformatics, geographical ecology, and the predictive modelling of global change impacts on biodiversity and ecosystems.

Paula Arribas is an entomologist at the University of Murcia (Spain) with a particular interest in the biogeography, ecology, evolution and conservation of beetles. Her research is currently focused on the fauna of saline waters from the Mediterranean region.

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