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Biogeografía del tritón ibérico (*Lissotriton boscai*): factores implicados en la diversidad interpoblacional

Biogeography of the Iberian newt (*Lissotriton boscai*): factors involved in interpopulation diversity

MEMORIA PARA OPTAR AL GRADO DE DOCTOR
PRESENTADA POR

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Memoria presentada por el licenciado Miguel Peñalver Alcázar para optar al grado de Doctor en Ciencias Biológicas, bajo la dirección del Doctor Pedro Aragón Carrera, del Museo Nacional de Ciencias Naturales.

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CONTENTS

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SUPPORTING INFORMATION

CHAPTER I

SUPPORTING INFORMATION

CHAPTER II

SUPPORTING INFORMATION

CHAPTER III

SUMMARY

Introduction

Understanding geographic variation in the characteristics of individuals and populations of species and/or closely related species is a long-standing research theme in biogeography and evolutionary biology. This endevour involves answering why species appear where they are; which are the factors driving species distributions; which is the relationship between the species and their environment; why closely related species or species populations share similar traits or environmental requirements; how species and species populations vary in space and time and which are the factors behind this heterogeneous response.

Research on biogeographical variation involves recognizing and describing patterns and unveiling the processes behind them. This entails some challenges, mainly because these patterns may arise from a plethora of factors and mechanisms that may act together, simultaneously or separately, at different spatial and temporal scales. The best approach to address this is combining different sources of information at different spatio-temporal ecological and evolutionary scales with multiple hypotheses.

Since the nineteenth century, many biogeographical patterns have been described at both the intraspecific and interspecific level. These patterns involve the co-variation of biological traits (e.g., body size) or phenomena (e.g., sexual size dimorphism, ecological niche) with geographical (e.g., latitude, altitude) and/or environmental (e.g., temperature, precipitation, primary productivity) variables.

The study of biogeographical patterns among species populations, rather than between species, is useful to explore different characteristics of the species evolutionary biology. Species populations experience heterogeneous environmental conditions along their distribution. This leads to the existence of geographical variation in species phenotypic and life-history traits, which may arise from genetic (e.g., local adaptation, divergent selection) and/or non-genetic mechanism (e.g., phenotypic plasticity). Additionally, comparing studies that include different populations throughout species geographical ranges is invaluable to draw generalities among species.

Currently there is an urgent need towards refining the knowledge of the causes and mechanisms behind intraspecific variation. This is especially true in the face of the rapid biodiversity declines, fuelled by global change components. Further insights into intraspecific variation among populations will not only improve the understanding of the species biogeography, ecology and evolution, but will also help to design conservation strategies and allocate efforts.

Objectives and Results

The general objective of this thesis is to deliver new, robust and integrated insight into the different and complex spatio-temporal factors and mechanisms behind intraspecific variation among populations. Therefore, we explore patterns and proccesses linked to geographical variation of body size, sexual size dimorphism (SSD) and environmental niche in the Iberian newt, *Lissotriton boscai*.

Chapters I and **II** target the study of patterns and processes of geographical body size and SSD variation among *L. boscai* populations. We address multiple and alternative non-mutually exclusive hypotheses related to physiological, ecological and reproductive characteristics of the species, integrating local and macro scale climatic and ecological factors with historical and contemporary (phylo)genetic information.

Specifically, in **Chapter I** we explore the pattern and processes of intraspecific geographical body size variation. We assess the differences and similarities of the influences of local and macro scale predictors on male and female body size among populations, while taking into account the phylogenetic relatedness of populations. This approach will help us to elucidate the role of genetic and non-genetic factors in geographical body size variation. We test eight leading hypotheses to reveal the potential mechanism(s) involved: heat conservation, water availability, converse water availability, primary productivity, endurance, seasonality, competition and/or predation, and temperature-size-rule. In **Chapter II**, the focus is on the pattern and processes of SSD. The research on this topic has been mainly conducted at interspecific level rather than at the intraspecific level. To fill this gap, we explore the interpopulation pattern of variation in SSD and its compliance with Rensch's rule or its inverse. We address four leading hypotheses to reveal the potential mechanism(s) involved: sexual selection, fecundity selection, density-dependent resource availability, and differential plasticity.

Finally, in **Chapter III**, we explore whether *L. boscai* intraspecific lineages respond homogeneously to the environmental conditions experienced by the species, throughout its distributional range. This allows us to assess to what degree responses vary and whether neglecting the existence of intraspecific variation may affect the overall performance, predictive capacity and transferability of Species Distribution Models. We also address the mechanisms potentially related to the ecological similarities or differences between the two intraspecific lineages, namely: niche conservatism and niche divergence.

The results of this thesis revealed that several mechanisms are responsible for the intraspecific variation among *L. boscai* populations. These mechanisms are sex and lineage-specific. Primary production, conspecific density and intraspecific competition, along with seasonality, are relevant for the geographical variation of body size and SSD (**Chapters I and II**). The relationship between SSD and body size of males and females follows the inverse of Rensch's rule, with female Iberian newts driving allometry in SSD (**Chapter II**). Subsequent analyses support the density-dependent resource availability hypothesis and the differential plasticity hypothesis, for explaining geographical variation in body size and SSD (**Chapters I and II**). Finally, primary production and its seasonality, along with temperature and precipitation seasonality, largely determine the differences between lineages in their geographical distribution and environmental niches, which are not identical (**Chapter III**).

Conclusions

1. The pattern of body size variation among *L. boscai* populations is related to sex-depended responses to climatic and ecological factors after accounting for the (phylo)genetic structure of populations.
2. Individuals in populations with higher primary productivity and lower annual temperature, or smaller fluctuations in primary productivity, present larger body sizes. Local conspecific density was negatively related to body size in males and females, but was the ecological factor with the strongest sex-bias. For females this predictor was among the three of greatest relevance, whereas for males it was among the three least important predictors

3. Female body size variation is mainly related to a process involving food availability, conspecific density and competition. This is in line with the hypothesis of density-dependent resource availability. In males, seasonal fluctuations in food availability was one of the most relevant predictors of body size variation, and several alternative non-exclusive hypotheses potentially underlie the pattern, namely: the heat conservation hypothesis, the temperature-size rule hypothesis and/or the seasonality hypothesis.

4. Interpopulation variation of SSD increased with increasing female mean body size, supporting the existence of the inverse Rensch's rule pattern in *L. boscai*.

5. Primary productivity-related variables and female density were among the most relevant ecological predictors of SSD after accounting for genetic structure and temporal autocorrelation. The interface between the density-dependent resource availability hypothesis and the differential plasticity hypothesis explains the inverse of Rensch's rule.

6. The two intraspecific phylogenetic lineages of *L. boscai* differ in their potential distribution and underlying environmental factors.

7. Intraspecific differences in predictive capacity and transferability of distribution models were detected independent of the inter-algorithm variability. The consideration of the intraspecific variation observed in *L. boscai* led to an improved intraspecific predictability of the lineage level based-distribution models.

8. *L. boscai* lineages showed a very low degree of niche overlap and occurred in relatively different environmental niches spaces (rejection of the null hypothesis of niche equivalence test). The existence of niche divergence or niche conservation could not be fully supported due to the non-rejection of the null hypothesis of niche identity test. There was partial spatial agreement between the environmental niche overlap and independently described geographical secondary contact zones between the lineages and/or clades of *L. boscai*.

9. Overall, primary productivity and seasonality of primary productivity, temperature and precipitation are among the most important macroscale predictors related to both interpopulational variation in body size, and hence to SSD, and the species and lineage geographical distributions and environmental niche.

RESUMEN

Introducción

La comprensión de la variación geográfica de las características de los individuos y las poblaciones de las especies o de especies estrechamente emparentadas constituye un tema de estudio inveterado en biogeografía y biología evolutiva. Esta tarea supone responder a por qué las especies aparecen donde están; cuáles son los factores responsables de la distribución de las especies; cuál es la relación entre las especies y su entorno; por qué las especies o poblaciones de especies estrechamente emparentadas comparten rasgos o requisitos ambientales similares; cómo las especies y las poblaciones de las especies varían en el espacio y el tiempo y cuáles son los factores que explican esta respuesta heterogénea.

La investigación sobre la variación biogeográfica implica el reconocimiento y la descripción de patrones y, a continuación, la dilucidación de los procesos subyacentes. Esto conlleva algunos retos, sobre todo porque dichos patrones pueden emerger como resultado de una plétora de factores y mecanismos que pueden actuar juntos, simultánea o independientemente, a diferentes escalas espaciales y temporales. El mejor enfoque para abordar esta cuestión consiste en combinar diferentes fuentes de información a diferentes escalas espacio-temporales, ecológicas y evolutivas con múltiples hipótesis.

Desde el siglo XIX, se han descrito distintos patrones biogeográficos tanto a nivel intraespecífico como interespecífico. Estos patrones implican la covariación de rasgos biológicos (p. ej., tamaño corporal) o fenómenos (p. ej., dimorfismo sexual, nicho ecológico) con variables geográficas (p. ej., latitud, altitud) o ambientales (p. ej., temperatura, precipitación, productividad primaria).

El estudio de patrones biogeográficos entre poblaciones de especies, más que entre especies, es útil para explorar diferentes características de la biología evolutiva de las especies. Las poblaciones de las especies experimentan condiciones ambientales heterogéneas a lo largo de su distribución. Esto motiva la existencia de variaciones geográficas en los rasgos fenotípicos y en las estrategias vitales de las especies que pueden derivarse de mecanismos genéticos (p. ej., adaptación local, selección divergente) o no genéticos (p. ej., plasticidad fenotípica). Además, la comparación de estudios que incluyen diferentes poblaciones a lo largo del área distribución geográfica de las especies es invaluable para trazar generalidades entre especies.

Actualmente existe una necesidad urgente de ahondar en el conocimiento de las causas y los mecanismos subyacentes a la variación intraespecífica. Esto es crucial frente a la rápida disminución de la biodiversidad debida a los diferentes componentes del cambio global. Un mejor conocimiento de la variación intraespecífica entre poblaciones no solo mejorará la comprensión de la biogeografía, la ecología y la evolución de las especies, sino que también ayudará al diseño de estrategias de conservación y a dirigir sus esfuerzos.

Objetivos y resultados

El objetivo general de esta tesis es ofrecer una perspectiva nueva, precisa e integral de los diferentes y complejos factores y mecanismos espacio-temporales que subyacen a la variación intraespecífica entre poblaciones. Para tal fin, examinamos patrones y procesos relacionados con la variación geográfica del tamaño corporal, el dimorfismo sexual en el tamaño corporal (DST) y el nicho ambiental en el tritón ibérico, *Lissotriton boscai*.

Los **Capítulos I y II** se centran en el estudio de los patrones y procesos de la variación geográfica del tamaño corporal y el DST entre poblaciones de *L. boscai*. Consideramos múltiples hipótesis alternativas no mutuamente excluyentes relacionadas con las características fisiológicas, ecológicas y reproductivas de la especie, integrando factores climáticos y ecológicos a escala local y macro con información (filo)genética histórica y contemporánea.

Específicamente, en el **Capítulo I** examinamos el patrón y los procesos de la variación geográfica intraespecífica en el tamaño corporal. Evaluamos las diferencias y similitudes de las influencias de los predictores a escala local y macro sobre el tamaño corporal de las hembras y los machos del tritón ibérico entre poblaciones, teniendo en cuenta las relaciones filogenéticas entre poblaciones. Este enfoque nos ayudará a dilucidar el papel de los factores genéticos y no genéticos en la variación geográfica del tamaño corporal. Evaluamos ocho hipótesis principales a fin de revelar los potenciales mecanismos involucrados: conservación del calor, disponibilidad de agua, inversa de la disponibilidad de agua, producción primaria, resistencia a la inanición, estacionalidad, competencia o depredación y regla de tamaño-temperatura. El **Capítulo II** se centra en el patrón y los procesos del DST. El estudio de esta cuestión se ha realizado principalmente a nivel interespecífico y no a nivel intraespecífico. Para colmar esta laguna, examinamos el patrón de variación interpoblacional del DST y su conformidad con la regla de Rensch o su inversa. Evaluamos cuatro hipótesis principales a fin de revelar los mecanismos potenciales involucrados: selección sexual, selección de fecundidad, disponibilidad de recursos dependiente de la densidad y plasticidad diferencial.

Finalmente, en el **Capítulo III**, investigamos si los linajes intraespecíficos de *L. boscai* responden homogéneamente a las condiciones ambientales experimentadas por la especie a lo largo de su rango de distribución. Esto nos permite evaluar hasta qué punto varían las respuestas y si pasar por alto la existencia de variaciones intraespecíficas podría afectar el rendimiento general, la capacidad predictiva y la transferabilidad de los Modelos de Distribución de Especies. También abordamos los mecanismos potenciales relacionados con las similitudes o diferencias ecológicas entre los dos linajes: conservación de nicho y divergencia de nicho.

Los resultados de esta tesis ponen de manifiesto que varios mecanismos son responsables de la existencia de variación intraespecífica entre las poblaciones de *L. boscai*. Estos mecanismos son específicos del sexo y del linaje. La producción primaria, la densidad de conespecíficos y la competencia intraespecífica, junto con la estacionalidad, son relevantes a la hora de explicar la variación geográfica del tamaño corporal y el DST (**Capítulos I y II**). La relación entre el DST y el tamaño corporal de los machos y las hembras sigue el patrón inverso de la regla de Rensch, siendo las hembras responsables de la existencia de alometría en el DST (**Capítulo II**). Los resultados de los diferentes análisis respaldan la hipótesis de disponibilidad de recursos dependiente de la densidad y la hipótesis de plasticidad diferencial a la hora de explicar la variación geográfica en el tamaño corporal y el DST (**Capítulos I y II**). Por último, la producción primaria y su estacionalidad, junto con la estacionalidad de la temperatura y la precipitación, determinan en gran medida las diferencias entre los linajes con respecto a su distribución geográfica y sus nichos ambientales, que no son idénticos (**Capítulo III**).

Conclusiones

1. El patrón de variación del tamaño corporal entre poblaciones de *L. boscai* está relaciona con respuestas dependientes del sexo a factores climáticos y ecológicos una vez considerada la estructura (filo)genética de las poblaciones.

2. Los individuos de poblaciones con mayor producción primaria y menor temperatura anual, o con menores fluctuaciones en la producción primaria, exhiben un mayor tamaño corporal. Aunque la densidad local de conespecíficos se relacionó negativamente con el tamaño corporal en machos y en hembras, fue el factor ecológico que exhibió un mayor sesgo entre sexos. En las hembras, este factor se encontraba entre los tres de mayor relevancia, mientras que en los machos se encontraba entre los tres predictores menos importantes.

3. La variación del tamaño corporal de las hembras está relacionado principalmente con un proceso que implica la disponibilidad de alimentos, la densidad de conespecíficos y la competencia intraspecífica. Esto concuerda con la hipótesis de que la disponibilidad de recursos depende de la densidad. En los machos, las fluctuaciones estacionales en la disponibilidad de alimentos fueron uno de los factores predictivos más relevantes de la variación del tamaño corporal y diferentes hipótesis alternativas no excluyentes pueden ser responsables del patrón observado, a saber: la hipótesis de la conservación del calor, la hipótesis de la regla tamaño-temperatura o la hipótesis de la estacionalidad.

4. La variación interpoblacional del DST aumentó con el aumento de la media del tamaño corporal de las hembras, lo que corrobora la existencia de un patrón inverso de la regla de Rensch en *L. boscai*.

5. Las variables relacionadas con la producción primaria y la densidad de hembras se encuentran entre los factores ecológicos más relevantes del DST tras considerar la estructura (filo)genética y la autocorrelación temporal de las poblaciones. La interfaz entre la hipótesis de disponibilidad de recursos dependiente de la densidad y la hipótesis de la plasticidad diferencial explicarían la regla inversa de Rensch.

6. Los dos linajes filogenéticos intraespecíficos de *L. boscai* difieren en su distribución potencial y en los factores ambientales subyacentes.

7. Se detectaron diferencias intraespecíficas en la capacidad predictiva y la transferibilidad de los modelos de distribución, independientemente de la variabilidad entre algoritmos. La consideración de la variación intraespecífica observada en *L. boscai* resultó en una mejor predictibilidad intraespecífica de los modelos de distribución basados en los linajes.

8. Los linajes de *L. boscai* exhiben un grado muy bajo de superposición de nicho y ocupan nichos ambientales relativamente diferentes (rechazo de la hipótesis nula de la prueba de equivalencia de nicho). La existencia de divergencia o conservación de nichos no pudo corroborarse plenamente debido a que no se rechazó la hipótesis nula de la prueba de identidad de nicho. Hubo una concordancia espacial parcial entre la superposición del nicho ambiental y las zonas de contacto secundario entre los linajes o clados de *L. boscai*, las cuales fueron descritas independientemente.

9. En conjunto, la producción primaria y la estacionalidad de la producción primaria, la temperatura y la precipitación se encuentran entre los factores a macroescala más importantes relacionados tanto con la variación interpoblacional del tamaño corporal y, por lo tanto del DST, como con la distribución geográfica y el nicho ambiental de la especies y los linajes.

•GENERAL INTRODUCTION•

GENERAL INTRODUCTION

Biogeography is focused on understanding the distribution of biological diversity in space and time by answering questions such as: Why do species appear where they are? Which are the bulk factors that rule species distributions? Which is the relationship between the species and their environment? Do closely related species or subspecies share similar traits or climatic and/or ecological requirements? How do species and species populations vary in space and time and which are the main factors underlying this heterogeneous response? (Brown & Lomolino, 1998). To address these questions, biogeography needs to embrace paradigms and research programs from different scientific disciplines including botany, zoology, ecology, evolutionary biology, genetics, phylogenetics, systematics and geography, among others. Biogeography is thus an interdisciplinary and multidisciplinary science, and further developments require a better integration of hypotheses, theories, data, methods and techniques (Wiens & Donoghue, 2004; Wen, Ree, Ickert-Bond, Nie & Funk, 2013). This complex and extensive endeavour will ultimately favour biodiversity conservation efforts in the face of the growing threat posed by the "Evil Sextet" —previously known as the "Evil Quartet", Diamond (1984)—, namely: over-exploitation, introduced invasive species and diseases, habitat loss and destruction, chains of linked extinctions, global climate change and extinction synergies (Brook, Sodhi & Bradshaw, 2008). These drivers of extinction are behind the current massive and rapid biodiversity loss (Wilson, 1985; Levin & Levin, 2002), acknowledged nowadays as the sixth big extinction (Pimm & Brooks, 2000; Wake & Vredenburg, 2008).

The main hallmark of biodiversity is its enormous interspecific diversity across all levels of organization, from the genetic and species levels to the ecosystem level (Gaston & Spicer, 1998). However, biodiversity also includes an intraspecific facet: the variation within species (Bolnick et al., 2011; Moran, Hartig & Bell, 2016). Naturalist and scientists have long been fascinated with the geographical variation in the characteristics of individuals and populations of species and/or closely related species (Gould & Johnston, 1972; Thorpe, 1987). Alexander Von Humboldt, Karl Bergmann, Alfred Russel Wallace and Charles Darwin observed and understood that animals and plants differed from place to place —for instance, at different altitudes and/or latitudes or within and among islands— and that this pattern of biological diversity across space was related to changes in physical, climate and/or environmental features. Thanks to this, they were able to propose groundbreaking theories that conform the heart of biogeography, ecology and evolutionary biology. For instance, by linking space and time in the notion of species variation, Wallace and Darwin laid the foundations for the theory of evolutionary change and the origin of species through natural selection (Darwin & Wallace, 1858).

The study of geographical variation first involves recognizing, documenting and describing broad and common regularities —i.e., patterns— and then disentangling the underlying ecological and evolutionary mechanisms —i.e., processes— at different temporal and spatial scales (Mayr, 1956; Lawton, 1999; Ricklefs, 2012). The most striking difference between patterns and processes is that while we can observe almost directly a pattern, the underlying process is sometimes hidden and has to be inferred. This obviously entails some challenges, mostly related to the multiple potential mechanisms driving a pattern (Grimm et al., 2005; Frank, 2009). However, successfully linking patterns and processes is crucial to develop ecological and evolutionary theories focused on improving the understanding of biodiversity and natural systems (Ashton, 2001).

Since the nineteenth century, biogeographers and ecologists have described many patterns at different spatial scales —from local to global scales— both at intraspecific and interspecific level. These regularities involve the co-variation of continuous or discrete biological traits (e.g., behavioural, morphological and ecological traits); different measures of biodiversity (e.g., richness, diversity and abundance of species); or phenomena and concepts (e.g., sexual size dimorphism, range size and ecological niche of species) with geographical (e.g., latitude, altitude) and/or environmental (e.g., temperature, precipitation, primary productivity) variables (Peterson & Holt, 2003; Blanckenhorn, Stillwell, Young, Fox & Ashton, 2006; Gaston, Chown & Evans, 2008; Holt, 2009). These biogeographical patterns "have come to be regarded" as biogeographical or ecogeographical "rules" or "laws" (Mayr, 1956; Gaston et al., 2008), including Bergmann's rule: increase in body size with latitude/altitude (Bergmann, 1847); Rensch's rule: sexual size dimorphism shows an allometric relationship with the body size of males and females among species (Rensch, 1950); Allen's rule: longer appendages in warmer environments (Allen, 1878); Rapoport's rule, increase in range size with latitude (Stevens, 1989); Steven's rule: increase of elevational range with elevation (Stevens, 1992); Cope's rule: body size in animal evolutionary lineages tends to increase over time (Cope, 1896); Foster's rule o Island rule: small species are larger on islands than on mainlands —gigantism—, whereas large species are smaller on islands than on mainlands —dwarfism (Foster, 1964).

The study of these biogeographical rules has attracted much attention and the number of papers published in the last years has increased dramatically. This tendency is very well illustrated through a literature search. Therefore, we performed a literature search in the Scopus database (https://www.scopus.com/; the largest abstract and citation database of peer-reviewed literature) for articles containing the words "Bergmann" and "body size" and "rule"; "Rensch" and "sexual dimorphism" and "rule"; "Allen" and "appendages" and "rule"; "Rapoport" and "rule"; "Foster" and "island" and "rule"; "Cope" and "body size" and "rule" in the title, abstract and/or keywords. A total of 1497 articles were identified spanning from 1948 to 2018 (articles from 2019 were excluded as the research in this year is still on course and therefore the records are incomplete). The data retrieved from Scopus revealed that research on biogeographical rules has increased exponentially between 1998 and 2018, with Bergmann's rule (644 articles), Rapoport's rule (180 articles) and Rensch's rule (151 articles) leading the trend (Fig. 1a). Moreover, Bergmann's rule is by far the most studied biogeographical rule from 1948 to 2018, with a 52% share of the studies (Fig. 1b).

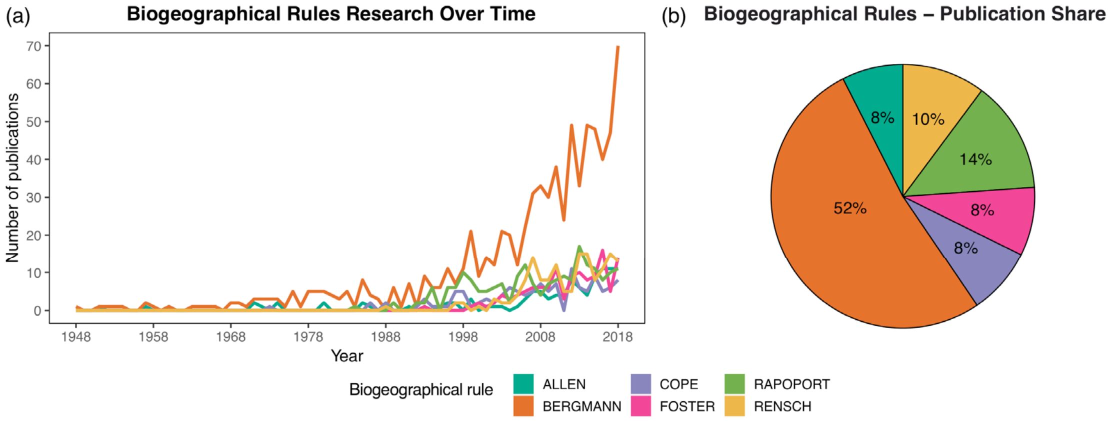

Figure 1 Literature search in the Scopus database. (**a**) Biogeographical rules research from1948-2018. (**b**) Biogeographical rules publication share from 1948-2018.

Ecological and evolutionary processes driving biogeographical patterns act and vary on spatial and temporal scales. Processes behind patterns at smaller scales —from micro and population scale to landscape scale— are suggested to act at a relatively faster pace than processes behind patterns at larger scales —from regional to continental and global scales. Therefore, it has been suggested that short-term ecological processes (e.g., biotic interactions, habitat features) drive patterns emerging over a smaller scales, whereas long-term evolutionary processes (e.g., lineages diversification and speciation) dominate patterns at larger scales (Brooks, 1988; Willis & Whittaker, 2002). However, the complexity of ecological systems arising from the inter-relationship, inter-action and inter-connectivity of their components renders that cross-scale interactions are commonplace when studying patterns in Nature. In this sense, patterns detected at a given scale of research may result from several mechanisms acting at different scales (scale up or scale down) other than the observed one (Levin, 1992; Gaston, 2000; Willis & Whittaker, 2002). More importantly, species involved in biogeographical patterns are the product of different ecological and evolutionary factors acting throughout their evolutionary history. Therefore, it is unlikely that a single and exclusive mechanism accounts solely for the existence of a given pattern (Mayr, 1956; Lawton, 1999; Lomolino, Sax, Riddle & Brown, 2006; Meiri, 2011).

The comprehensive research on biogeographical patterns has witnessed an intense and long lasting debate regarding their validity, underlying processes and level of applicability (i.e., intraspecific or interspecific level, endotherms or ectotherms, vertebrates or invertebrates), as well as the extent to which they can be regarded as rules or general laws in nature based on their generality (Scholander, 1955; Mayr, 1956; Lawton, 1999; Ashton, 2001; Lomolino et al., 2006; Gaston et al., 2008; Watt, Mitchell & Salewski, 2010; Olalla-Tárraga, 2011; Watt & Salewski, 2011). The more sceptical view arises as a result of evidence for some of the patterns being scarce, fragmented and only holding for specific group of animals (e.g., Bergmann’s rule, Cope’s rule, Rapoport's rule), with exceptions sometimes being abundant, depending on the pattern, organisms and level of biological organization under study (Gaston, Blackburn & Spicer, 1998; Blackburn, Gaston & Loder, 1999; Gaston et al., 2008). Additionally, sceptics may argue that pattern testing should be strictly restricted to the exact level of biological organization (e.g., intraspecific, interspecific), group of animals (e.g., endotherms, ectotherms) and/or mechanism proposed in the original rule (Watt et al., 2010; Salewski & Watt, 2017). This train of thought lead to establish that pattern and process are indistinguishable and intimately interwoven, so a pattern cannot to be proven (or exist, for that matter) if the inherent process is not supported. For the advocates of a more "pluralistic" view, however, exceptions are in fact expected to occur. Under this view, the common criterion for a pattern to constitute a generality is that it is displayed by more than of 50% of the species studied (Mayr, 1956; Ashton, 2001). Moreover, the pattern holds even when the process driving the pattern proves to be different than the one original proposed (Meiri, 2011; Olalla-Tárraga, 2011), as biological and environmental problems faced by species throughout their evolutionary history may have (and more often do than not) multiple solutions (Mayr, 1956). These arguments thus tend to promote not only a less restrictive approach on the pattern and process relationship, but also the need to clearly distinguish between both elements. Finally, the "pluralistic" view supports expanding the research program on biogeographical patterns to other groups of organisms such as lizards, amphibians, and insect to overcome taxonomic bias towards mammals and birds, and increasing studies at intraspecific level. The rationale is that different groups of organisms with contrasting ecological and evolutionary histories may have developed specific solutions to cope with biological, environmental and climatic conditions (Lomolino et al., 2006; Gaston et al., 2008).

The study of biogeographical patterns at the intraspecific level, rather than at the interspecific level, provides a better approach to explore different characteristics of the species evolutionary biology. Species populations are exposed to heterogeneous climatic and environmental conditions along their distributional range, which ultimately leads to the existence of geographical variation in species phenotypic and life-history traits (Roff, 1992). This intraspecific variation may arise from genetic (i.e., local adaptation, divergent selection or incipient speciation) and non-genetic mechanisms (i.e., phenotypic plasticity), or both (e.g., Via & Lande, 1985; Stearns, 1989; Sorci, Clobert & Belichon, 1996; Miaud & Merilä, 2001; Sears & Angilletta, 2003; Kawecki & Ebert, 2004). For example, on the one hand organisms can respond to local environmental factors such as abiotic (e.g., temperature, precipitation or length of the growing season) and biotic (e.g., intraspecific competition) factors through long-term adaptation. Therefore, they should exhibit a higher fitness in their local habitat, and could be negatively affected under new environmental conditions (Olsson & Uller, 2003; Leimar, Doebeli & Dieckmann, 2008; Gomez-Mestre, Kulkarni & Buchholz, 2013). On the other hand, organisms can respond via phenotypic plasticity —defined as the ability of an individual genotype to exhibit different phenotypes in response to different environmental conditions. This allows them to develop a phenotype matching the environmental conditions and therefore to maximise their fitness (Agrawal, 2001; Ernande & Dieckmann, 2004; Miner, Sultan, Morgan, Padilla & Relyea, 2005).

Determining whether intraspecific geographical variation in species traits arises from local adaptation and/or phenotypic plasticity is important not only to improve the understanding of biogeographical patterns, but also to predict the species impact on ecosystems and their responses to global change (Stillwell, 2010; Moran et al., 2016; Des Roches et al., 2018). Notwithstanding, conclusions derived from field studies have to be taken with caution because it is difficult to separate local adaptation from phenotypic plasticity in natural populations (Stillwell & Fox, 2009). The interesting thing about examining spatial (geographical) variation at the intraspecific level is that it connects us with evolutionary processes in a way that is accessible to the human observer. The alternative, examining temporal variation, is not available to the human observer whose time scale does not match the time scale at which evolution usually occurs. Additionally, intraspecific studies including populations throughout species geographical ranges are valuable to prove generalities among species, by comparing results obtained in different species. Moreover, patterns and processes at the intraspecific level do not necessarily match those at the interspecific level. Overall, intraspecific studies allow us to identify and explore exceptions in different organisms and levels of biological organization. This will provide further insight into the multiple and alternative mechanisms (hypotheses) at play, thereby building consensus on biogeographical patterns and improving ecological and evolutionary knowledge of biodiversity (Lomolino et al., 2006; Gaston et al., 2008).

Pattern and processes of body size variation

Body size is one of the most relevant characters of animal species and it is related to several physiological, ecological and life history traits such as lifespan, fecundity, developmental time, diet width and species richness. Therefore body size has broad implications not only for species ecology and evolution, but also for ecosystems dynamics (Roff, 1992; Woodward et al., 2005). Geographical patterns of variation in body size have fascinated biologist since the nineteenth century, following the pioneering work of Karl Bergmann (Bergmann, 1847; partial translation in James, 1970 and; Watt et al., 2010) and the resulting establishment of the most well-know ecogeographical pattern, the so-called Bergmann's rule (Blackburn et al., 1999; Ashton, 2001; Millien et al., 2006; Vinarski, 2014). This rule predicts a negative relationship between body size and temperature. Specifically, the body size of individuals within the geographical distribution of a species is predicted to increase at higher latitudes or altitudes under colder climatic conditions and decrease at lower latitudes or altitudes under warmer climatic conditions. The adaptive mechanism proposed to this pattern is related to the heat conservation quantity of surface area to volume ratio: larger animals have a small surface area to volume ratio and therefore they lose heat at a lower rate (e.g., Mayr, 1956). The original scope of Bergmann’s rule was to describe geographical body size variation among endothermic species within a genus (Blackburn et al., 1999; Watt et al., 2010). Later on the rule was reformulated by Rensch (1938) and Mayr (1956), extending its applicability to populations within species.

Geographic body size variation has been investigated at interspecific and intraspecific levels both in endotherms and ectotherms. In endothermic vertebrates, there is a consensus on the existence of a “Bergmannian size pattern” for mammals (Ashton, Tracy & de Queiroz, 2000; Freckleton, Harvey & Pagel, 2003; Meiri & Dayan, 2003; Rodríguez, Olalla-Tárraga & Hawkins, 2008) and birds (Ashton, 2002a; Meiri & Dayan, 2003; Olson et al., 2009). Notwithstanding, the validity of this pattern and the generality of the original underlying mechanism of heat conservation have been long criticized (e.g. Scholander, 1955; McNab, 1971; Geist, 1987). For ectothermic vertebrates such anurans and urodeles, the generality of this trend is more complex, with studies supporting it (Ashton, 2002b; Olalla-Tárraga & Rodríguez, 2007; Ficetola et al., 2010; Slavenko & Meiri, 2015; Amado, Bidau & Olalla-Tárraga, 2019), studies documenting the existence of an inverse of such a trend (Olalla-Tárraga & Rodríguez, 2007; Adams & Church, 2008; Cvetković, Tomašević, Ficetola, Crnobrnja-Isailović & Miaud, 2009; Slavenko & Meiri, 2015) and studies suggesting that amphibians do not follow any pattern at all (Adams & Church, 2008). The work within this biological group has led to additional hypotheses other than thermoregulation underlying body size variation (Ashton, 2002b; Gouveia & Correia, 2016).

Recent years have witnessed an intense debate between scientists about whether testing Bergmann's rule should be strictly restricted to the interspecific level, among endothermic species, and to its inherent mechanism (Watt et al., 2010; Meiri, 2011; Olalla-Tárraga, 2011; Watt & Salewski, 2011). Watt et al. (2010) proposed that Bergmann's rule should be tested only under its original formulation —i.e., interspecific variation in endothermic vertebrates—, and highlighted the need to experimentally demonstrate the heat conservation mechanism behind Bergmann's rule rather than focusing on the existence or not of the pattern itself. Meanwhile, other researches claim that the intraspecific notion of Bergmann's rule was implicit in its original postulate and that the pattern should be equally applicable at intraspecific and interspecific levels. Moreover, even though ectotherms use external heat sources to regulate their body temperature —i.e., the relationship between body size and heat conservation may be less clear than in endotherms—, the thermoregulatory explanation for body size clines in ectotherms should not be totally dismissed (Meiri, 2011; Olalla-Tárraga, 2011; Zamora-Camacho, Reguera & Moreno-Rueda, 2014). Finally a clear distinction should be made between the observed pattern and the processes behind it. Olalla-Tárraga (2011) argued in his reply to Watt et al. (2010), similar to Mayr´s (1956) previous reply to Scholander (1955), that failing to find evidence in support of a proposed mechanism does not invalidate the pattern itself. This is because several mechanisms, rather than one single and exclusive mechanism, may account for the existence of a given pattern. The interdependence between ecosystem components suggests that several mechanisms may act individually, synergically and/or in opposite directions, as well as operate on different temporal and/or spatial scales, reflecting the myriad of ecological factors and different selective (evolutionary) forces acting on the species (Mayr, 1956; Lawton, 1999; Lomolino et al., 2006; Meiri, 2011).

The contrasting results in this extensive research field thus render that a simple and unified explanation of geographical body size variation in ectotherms and endotherms is far from within reach. Currently, the view held by the majority of the researches is that several ecological, environmental and climatic factors —including temperature, precipitation, moisture content and primary productivity, among others— are responsible for the observed “Bergmannian size patterns” (James, 1970; Blackburn & Hawkins, 2004; Yom-Tov & Geffen, 2006; Stillwell & Fox, 2009). However, these factors are commonly intercorrelated. Therefore understanding the processes behind geographical body size variation implies testing multiple non-exclusive hypotheses simultaneously to shed light on the relative contribution of several ecological and/or physiological mechanisms (Ashton et al., 2000; Olalla-Tárraga & Rodríguez, 2007). At present the main hypotheses for explaining geographical body size variation in ectothermic and endothermic species are:

- **Heat conservation:** larger endothermic animals exhibit a lower surface to volume ratio and, consequently, engage in more effective thermoregulation, with lower heat loss, in environments with colder temperatures (Bergmann, 1847; Mayr, 1956). For ectothermic animals such as amphibians, it implies that those species with thermoregulatory capacity (mostly anurans) would tend to be larger in colder environments, while thermoconformers (urodeles) would tend to be smaller in colder environments (Fig. 2a,b; Olalla-Tárraga & Rodríguez, 2007).
- **Resource availability or primary productivity:** predicts that body size should respond to variations in resource availability. Therefore body size increases in regions with higher resource availability or primary productivity (Fig. 2c; Rosenzweig, 1968; Yom-Tov & Geffen, 2006; Huston & Wolverton, 2011).
- **Water availability:** James (1970) proposed that moisture may also play a significant role in geographical body size variation. The rationale is that surface to volume ratio also influences water balance, such that larger individuals have greater dessication tolerance resulting from a relative decrease in surface area. The availability of water or moisture can be of great importance for animals living in hot and drier environments (Burnett, 1983) or for those intimately linked to the aquatic environment, such as amphibians (Fig. 2d; Ashton, 2002b; Gouveia & Correia, 2016).
- **Converse water availability:** animals attain larger body size in areas with greater precipitation. For amphibians this is especially relevant as their pattern of activity is tightly linked to high water availability or periods with high humidity (Fig. 2e; Ficetola et al., 2010).
- **Endurance:** larger body size is favoured in colder environments with a high seasonality as larger size leads to greater fat reserves, allowing individuals to cope with (longer) food shortage periods (Fig. 2f; Boyce, 1979; Lindstedt & Boyce, 1985; Ashton, 2002b).

- **Seasonality:** larger body size is favoured in areas with a longer and homogeneous growing season (longer activity period), while smaller body size is favoured in areas with a shorter and heterogeneous growing season (Fig. 2g; Boyce, 1979; Lindstedt & Boyce, 1985; Mousseau, 1997).
- **Competition and/or predation:** body size of species tends to deviate from their optimal under competition and predation (Damuth, 1993). Accordingly, larger body size results from reduced biotic relationships —i.e., reduced richness or abundance of intra and interspecific competitors and predators (Fig. 2h; Ashton & Feldman, 2003; Desdevises, Legendre, Azouzi & Morand, 2003; Olson et al., 2009).
- **Temperature size rule:** development and growth rates are lower in colder environments, leading to longer embryonic development and growth periods, which result in delayed maturity and larger adult body size (Fig. 2i; Atkinson & Sibly, 1997; Angilletta & Dunham, 2003).

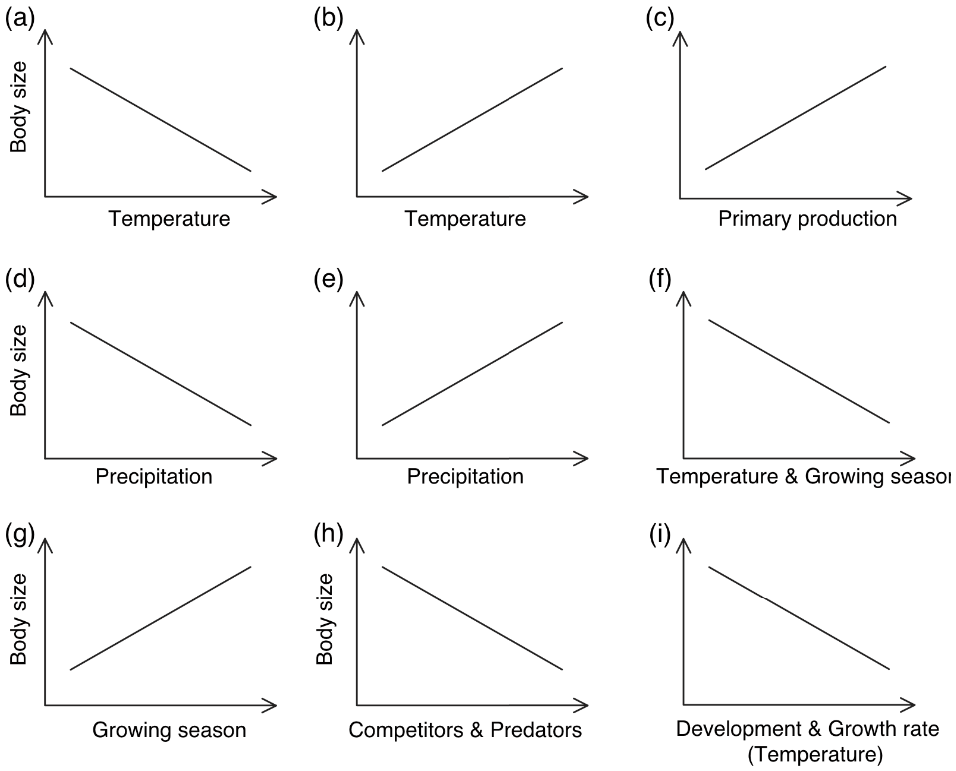

Figure 2 General relationships between body size and predictors for the different hypotheses explaining geographical body size variation. (**a**) Bergmann's rule (heat conservation). (**b**) Converse Bergmann's rule. (**c**) Resource availability. (**d**) Water availability. (**e**) Converse water availability. (**f**) Endurance. (**g**) Seasonality. (**h**) Competition and/or Predation. (**i**) Temperature size rule.

Pattern and processes of sexual size dimorphism

Males and females of animal species frequently differ in their body size, with one sex being larger than the other, a phenomenon referred as sexual size dimorphism (SSD; Andersson, 1994). Females are usually larger than males (female-biased SSD) in amphibians, fish and insects, whereas male-biased SSD is widespread in birds, mammals and lizards (Fairbairn, Blanckenhorn & Székely, 2007). The German evolutionary biologist Bernhard Rensch observed the existence of an allometric relationship between sexual size dimorphism (SSD considered as size of the larger sex/size of the smaller sex) and body size of males and females among species. The so-called Rensch's rule poses that SSD increases with body size when males are larger than females —in species with male-biased SSD— and that SSD decreases with body size when females are larger than males —in species with female-biased SSD (Fig. 3; Rensch, 1950; Abouheif & Fairbairn, 1997; Fairbairn, 1997). Moreover, studies have found patterns in support of an inverse of Rensch's rule: the level of female-biased SSD increases with increasing female mean size in female-biased SSD species where females are the more variable sex in terms of body size (Fig. 3; e.g., Herczeg, Gonda & Merilä, 2010; Liao, Liu & Merilä, 2015). Rensch originally established that this trend applies to subspecies, genus and/or family levels, namely at the interspecific level (Abouheif & Fairbairn, 1997). However, the scope of Rensch' rule has recently shifted to the intraspecific level (Fairbairn, 2005; Blanckenhorn et al., 2006; Lengkeek et al., 2008).

Rensch's rule has turned out to be a widespread biogeographical pattern, and has been mainly studied at the interspecific level for several and different animal taxa, including shorebirds, humming birds, lizards, salamanders, water striders, dragonflies and mites (Fairbairn, 1997; Colwell, 2000; Cox, Skelly & John-Alder, 2003; Székely, Freckleton & Reynolds, 2004; Johansson, Crowley & Brodin, 2005; Colleoni, Denoël, Padoa-Schioppa, Scali & Ficetola, 2014). Studies at the intraspecific level are slowly emerging as well, although they are still way behind compared to those at interspecific level (Fairbairn, 2005; Blanckenhorn et al., 2006; Lengkeek et al., 2008). Support for Rensch's rule has been commonly found in male-biased SSD species, for both endotherms and ectotherms at the interspecific level (e.g., Fairbairn, 1997; Cox et al., 2003; Székely et al., 2004; Webb & Freckleton, 2007). However, the support for the rule does not hold for female-biased SSD endotherms and ectotherms at the interspecific level (e.g., Webb & Freckleton, 2007; Liao, Zeng, Zhou & Jehle, 2013). Interestingly, intraspecific studies in ectotherms usually show the existence of the inverse of Rensch's rule (Herczeg et al., 2010; Liao et al., 2015). In amphibians, the study of the validity of Rensch Rule or its inverse, both at inter and intraspecific levels, has recently attracted considerable attention. These studies have provided contrasting results either supporting the existence of a relationship between SSD and male body size consistent with Rensch's rule (urodeles, interspecific level; Colleoni et al., 2014), the existence of a relationship between SSD and female body size consistent with the inverse of Rensch's rule (anurans and urodeles, inter and intraspecific levels; Ivanović, Sotiropoulos, Furtula, Džukić & Kalezić, 2008; De Lisle & Rowe, 2013; Liao, 2013; Liao et al., 2015) or the absence of any relationship between SSD and male and/or female body size (anurans and urodeles, inter and intraspecific levels; Liao et al., 2013; Colleoni et al., 2014; Nali, Zamudio, Haddad & Prado, 2014; Sinsch, Pelster & Ludwig, 2015). Overall, results appear to depend on the phylogenetic level and species group, leading to an unclear pattern of variation of SSD in amphibians.

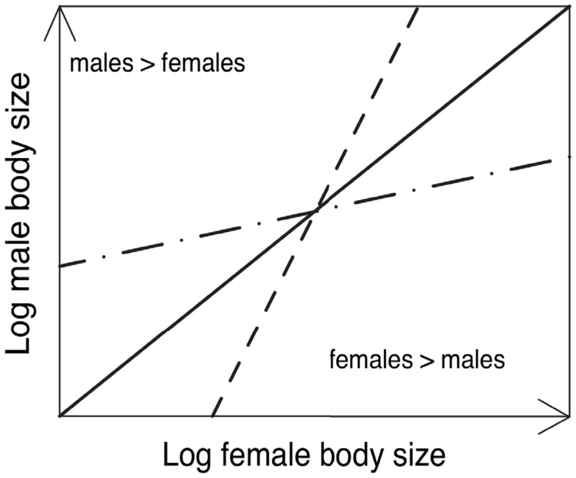

Figure 3 Schematic representation of Rensch's rule and its inverse as predicted by the regression model commonly used for estimating allometry for sexual size dimorphism (SSD), with the logarithm of male body size on the y-axis and the logarithm of female body size on the x-axis (Fairbairn, 1997). The diagonal solid line represents isometry, i.e., male body size = female body size. The dotted line represents an allometric relationship in which male body size varies more among species (or populations) than female body size, leading to a regression slope of $\beta > 1$. This relationship is consistent with Rensch's rule, with SSD increasing with body size in male-biased SSD species and decreasing with body size in female-biased SSD species. The dot-dashed line represents an allometric relationship in which female body size varies more among species (or populations) than male size and therefore the regression slope is $\beta < 1$. This relationship is consistent with the inverse Rensch's rule, with SSD increasing with body size in female-biased SSD species and decreasing with body size in male-biased SSD species. Figure adapted from Fairbairn (1997).

The exact mechanisms driving SSD and patterns conforming to Rensch's rule or its inverse remain unclear, with behavioural, ecological, physiological, reproductive and sexual factors playing a significant role, among others (e.g., Shine, 1979; Slatkin, 1984; Shine, 1989; Blanckenhorn, 2005). The prevalent view is that sexual selection in males and fecundity selections in females are the main evolutionary mechanisms leading to SSD at inter and intraspecific levels (Abouheif & Fairbairn, 1997; Fairbairn, 1997; Cox et al., 2003; Dale et al., 2007; García-Navas, Bonnet, Bonal & Postma, 2016). However, this assumption is open to exceptions (Shine, 1988; Clutton-Brock, 2009; Pincheira-Donoso & Hunt, 2017). Additionally, other mechanisms including sex-specific phenotypic plasticity (Fairbairn, 2005; Lengkeek et al., 2008; Rohner, Teder, Esperk, Lüpold & Blanckenhorn, 2018) or responses to resource availability and uptake (Colwell, 2000; Teder & Tammaru, 2005) can lead to intraspecific variation in SSD. The main leading hypotheses behind the SSD and Rensch's rule or its inverse can be summarised as follows:

- **Sexual selection hypothesis:** sexual selection acts more strongly in one sex, followed by a simultaneous but weaker correlational selection in the other sex (Abouheif & Fairbairn, 1997; Fairbairn, 1997). Sexual selection favours larger male body size in male-biased SSD species, generally because larger males exhibit a greater reproductive success (Andersson, 1994). However, sexual selections favours smaller male body size in female-biased SSD species because smaller body size confers better agility and manoeuvrability —an advantage for species with complex behavioural courtship displays performed in a three-dimensional space, such as water or air in swimming or flying species (Székely et al., 2004; Serrano-Meneses, Córdoba-Aguilar, Azpilicueta-Amorín, González-Soriano & Székely, 2008). Similarly, sexual selection may favour larger female body size because larger size provides an advantage during intrasexual competition for breeding opportunities or access to mates (Clutton-Brock, 2007, 2009). Overall, sexual selection acting on male body size is expected to result in a pattern consistent with Rensch's rule, i.e., SSD increases with body size in male-biased SSD species, while sexual selection acting on females should results in the inverse Rensch's rule, i.e., SSD increases with body size in female-biased SSD species.

- **Fecundity selection hypothesis:** there is a positive correlation between female body size and parameters related to female reproductive investment such as egg size and clutch size. Therefore, larger females are able to produce larger clutches or eggs, and ultimately more and larger offsprings. Fecundity selection, or the "fecundity advantage model", was originally proposed by Darwin (1874) to explain why females are larger than males in female-biased SSD species (Shine, 1988). Under this hypothesis, SSD increases with body size in female–biased SSD species and decreases with body size in male- biased SSD species, leading to a pattern consistent with the inverse Rensch's rule (e.g., Fairbairn, 1997; Herczeg et al., 2010; Liao, 2013). In most ectotherms, and especially in amphibians, the number of eggs produced by females usually increases with body size, independent of the reproductive mode (e.g., from aquatic reproduction with unprotected eggs and larvae to terrestrial reproduction with protected eggs and larvae, along with a intermediate modes), thus supporting the existence of female-biased SSD based on fecundity selection (Gomez-Mestre, Pyron & Wiens, 2012). However, in those species where clutch size does not increase with female size (Kupfer, 2007), female-biased SSD is unlikely to be the result of fecundity selection.
- **Differential plasticity hypothesis:** SSD can vary among populations with contrasting climatic, environmental and ecological conditions due to differences in phenotypic plasticity between males and females (Fairbairn, 2005). Under this hypothesis, if males show greater phenotypic plasticity than females, SSD would evolve following a pattern consistent with Rensch's rule. If, instead, females show greater phenotypic plasticity than males, SSD would evolve following a pattern consistent with the inverse Rensch's rule (Fairbairn, 2005; Rohner et al., 2018). Research into the impact of sex-specific phenotypic plasticity on SSD is recently new, yet different studies show that phenotypic plasticity is a relevant contributor to intraspecific SSD variation (Lengkeek et al., 2008; Stillwell & Fox, 2009). Although the reasons why males or females show greater plasticity are not yet fully understood, their different reproductive roles may hold an answer (Stillwell, Blanckenhorn, Teder, Davidowitz & Fox, 2010). For example, males and females commonly differ in nutrient requirements and energy allocation in reproduction, with females usually allocating more energy to

offspring or/and egg production (Shine, 1989; McNab, 2006). This phenomenon is linked to the last hypothesis.

- **Density-dependent resource availability hypothesis:** the differences between males and females in nutritional requirements and energy allocation in mating and reproduction could result in sex-specific responses of body size to environmental variation in resource availability and conspecific density (Colwell, 2000; Green & Middleton, 2013). Therefore, resource limitation may be a key driver of SSD patterns under high population density (Garel, Solberg, SÆther, Herfindal & Høgda, 2006). Higher nutritional constraints in females, relative to males would lead to a SSD pattern consistent with the inverse Rensch's rule, whereas higher constraints in males would lead to a SSD pattern consistent with Rensch's rule.

Pattern and processes of species distribution

Why species or closely related species are distributed where they are and which abiotic and biotic factors are driving species distributions are fundamental questions in biogeography and ecology. Its answer will result in an improved understanding of species ecology and evolution and will favour their conservation (Guisan & Zimmermann, 2000; Guisan & Thuiller, 2005).

The last decades have witnessed an exponential use of species distribution models (SDMs) —also know as ecological niche models (ENMs), habitat suitability models (HSMs) or habitat distribution models (HDMs) (Franklin, 2010; Guisan et al., 2013; Brotons, 2014). This growing interest in modelling species distribution and environmental requirements has come hand in hand with the development of new and refined modelling techniques and the increasing availability and accessibility of biodiversity and environmental databases (Graham, Ferrier, Huettman, Moritz & Peterson, 2004; Guisan et al., 2006b; Kozak, Graham & Wiens, 2008). SDMs provide detailed predictions on species geographical distributions and habitat suitability by relating species occurrence records (presence, presence/absence and/or abundance) to several climatic, environmental and biological variables (Franklin, 2010). The use of SDMs allows researchers, conservation planners, natural resource managers and policy makers to address a wide array of fundamental and applied issues in ecological and evolutionary biogeography, as well as in conservation and resource management and planning. Regarding fundamental questions, SDMs can be used for quantifying and characterizing the environmental niche of species (Warren, Glor & Turelli, 2008; Broennimann et al., 2012), testing evolutionary hypotheses in biogeography (Peterson, Soberón & Sánchez-Cordero, 1999; Wiens & Graham, 2005) and predicting species characteristics and population density (Vanderwal, Shoo, Johnson & Williams, 2009; Lunghi et al., 2018). Regarding applied questions, SDMs can be used for assessing the impact of climate change on biodiversity (Thomas et al., 2004; Aragón, Rodríguez, Olalla-Tárraga & Lobo, 2010a; Araújo & Peterson, 2012), improving biodiversity discovery and sampling (Guisan et al., 2006a), assessing risk and impact of biological invasions (Jiménez-Valverde et al., 2011; Wang & Jackson, 2014; Chapman, Scalone, Štefanić & Bullock, 2017) and reserve and conservation planning (Ferrier, 2002; Wilson, Westphal, Possingham & Elith, 2005; Araújo, Lobo & Moreno, 2007), among other applications.

Several concerns have been raised in relation to the limitations and uncertainty of SDMs and their predictions (Thuiller et al., 2004). These limitation and uncertainties include: (**a**) SDMs usually fail to include biotic interactions, thus ignoring a relevant phenomenon that shapes species ecology and evolution (Araújo & Luoto, 2007; Austin, 2007; Aragón & Sánchez-Fernández, 2013); (**b**) SDMs assume that species are at (quasi) equilibrium with the environmental conditions within a given region/area. In other words, species are present in all climatically suitable areas and absent from all unsuitable ones (e.g., Araújo & Pearson, 2005; Guisan & Thuiller, 2005); (**c**) The decision for or against a certain SDM algorithm or technique has a potential impact on the final findings and conclusions. SDMs usually differ in the type of response variables used (e.g., presence, presence/absence, abundance), the type of predictors that they can handle (categorical, continuous, or both), how they summarize the relationship between the response and predictor variables, their output (e.g., probabilities, continuous indices of habitat suitability), their ability to transfer model predictions in space and time and their predictive performance, among many others (e.g., Guisan & Thuiller, 2005; Randin et al., 2006; Austin, 2007; Elith & Graham, 2009); (**d**) SDMs commonly treat a species as a single unit, neglecting the existence of (phylogenetic and/or phenotypic) intraspecific variation within a species and assuming that its response to climatic and environmental conditions is always uniform across its distribution range. However, the response of species populations or lineages to the climatic and environmental conditions they experienced often varies in space and/or time (Valladares et al., 2014; Smith, Godsoe, Rodríguez-Sánchez, Wang & Warren, 2019). Therefore, species population and phylogeographic structure should be taken into account when modelling species distributions and niche structure (Pearman, D'Amen, Graham, Thuiller & Zimmermann, 2010), especially in those species with a marked phylogeographic structure (D'Amen, Zimmermann & Pearman, 2013).

As we have seen previously, species morphological, behavioural, physiological characters and life-history traits exhibit variation along latitudinal, climatic and environmental clines (Lomolino et al., 2006; Moran et al., 2016). Moreover, species show "intraspecific geographic variation in their niches" (Peterson & Holt, 2003; Holt, 2009). The phylogeographic structure within a species may represent a defined and distinct niche space among species lineages. Lineage-specific niche space may inform on the species evolutionary capacity to cope and/or adapt to contrasting climatic and ecological conditions (Wiens & Donoghue, 2004; Wiens & Graham, 2005). Understanding how species or intraspecific lineages respond to the environmental conditions over evolutionary time-scales is key to improve the ecological and evolutionary knowledge of the existing biodiversity. The response to environmental conditions can be conserved or divergent from species to species —or intraspecific lineages. Niche conservatism is the tendency of closely related species or evolutionary lineages to retain ancestral ecological adaptations, so their ecological differences are lower than expected by chance (Peterson et al., 1999; Wiens & Graham, 2005; Warren et al., 2008). On the other hand, niche divergence is the tendency of closely related species or evolutionary lineages to exhibit divergent natural selection in response to contrasting ecological conditions, so their ecological differences are greater than expected by chance (Warren et al., 2008; Schluter, 2009). These two concepts are intimately related with the biogeography of speciation: niche conservatism is usually related to allopatric speciation under similar ecological conditions/niches (Peterson et al., 1999; Wiens & Graham, 2005), while niche divergence is usually related to sympatric and parapatric speciation (Via, 2001), but also to allopatric speciation if diverging lineages inhabit geographical areas with contrasting environments (Wiens & Graham, 2005; Kozak & Wiens, 2006).

Overall, including intraspecific variation in SDMs is a relevant challenge to improve the overall performance and prediction ability of SDMs (Zimmermann, Edwards, Graham, Pearman & Svenning, 2010; Gonzalez, Soto-Centeno & Reed, 2011). Modelling the distribution and ecological niches of phylogenetic intraspecific lineages with a marked geographical structure may provide relevant information on each lineage that could be neglected when modelling the whole species. Ultimately, this will lead to a better evolutionary and ecological understanding of the species (Pearman et al., 2010; D'Amen et al., 2013). The increasing availability of genetic and ecological data coupled with the continuous refinement of modelling and statistical tools (Guisan et al., 2006b; Kozak et al., 2008) has helped to make increasingly feasible a phylogenetic-informed species distribution-modelling approach (e.g., McCormack, Zellmer & Knowles, 2010; Pearman et al., 2010; Fitze et al., 2011; Smith et al., 2019).

•OBJECTIVES•

OBJECTIVES

The general objective of this thesis is to provide new, robust and integrated knowledge on the different and complex spatio-temporal factors and mechanisms underlying variation within species. This knowledge is fundamental not only to advance the biogeographical, ecological and evolutionary understanding of biodiversity, but also to guide conservation efforts in the face of the current and future climatic and environmental change. To achieve this aim, we study the geographical variation of body size, sexual size dimorphism and environmental niche using the Iberian newt, *Lissotriton boscai*, as a model species (see General Methods section for a detailed justification on the selection of the study species). With this broad framework in mind, we explicitly address the following specific objectives:

In **Chapters I** and **II** we set out to study the patterns and processes of geographical body size and sexual size dimorphism variation in several populations representative of the environmental, phylogenetic and geographic range of *L. boscai*. We address multiple and alternative non-mutually exclusive hypotheses related to physiological, ecological and reproductive characteristics of the species that are potentially involved in the intraspecific geographical variation of the characters under study. To do so, we combine climatic and ecological factors at different spatial scales —from local scale to macro scale— with (phylo)genetic information at different temporal scales —from historical to contemporary evolutionary events. This integrated and multiscale approach allows us to explore past and current processes driving the observed patterns.

Specifically, in **Chapter I** we assess the pattern and processes of intraspecific geographical variation in body size. In contrast to other studies, we address this by exploring differences and similarities of the influences of local and macro scale predictors on male and female body size among populations, while accounting for population genetic structure. This allows us to address the possible role played by different genetic and non-genetic factors in the observed spatial and temporal variation. We evaluate eight leading hypotheses to reveal the potential mechanism(s) involved, namely: heat conservation, water availability, converse water availability, primary productivity, endurance, seasonality, competition and/or predation, and temperature-size-rule.

In **Chapter II**, we focus on the pattern and processes of sexual size dimorphism (SSD). To date, the majority of the research in this field has been conducted at interspecific level rather than at the intraspecific level. Moreover, intraspecific studies in amphibians are still scarce, especially in urodeles. Therefore, we set out to bridge this knowledge gap by studying the interpopulation pattern of variation in SSD and its compliance with Rensch's rule. For this, we explore whether the relationship between SSD and body size of males and females follows Rensch's rule or its inverse. Then we address four leading hypotheses to reveal the potential mechanism(s) involved, namely: sexual selection, fecundity selection, density-dependent resource availability, and differential plasticity. Based on the findings revealed by the study of geographical body size variation (**Chapter I**), we expect that sex-specific plasticity in body size might be responsible, at least partially, for the intraspecific pattern of variation in SSD in *L. boscai*.

In **Chapter III**, we investigate whether divergent populations or intraspecific lineages of *L. boscai* respond homogeneously over space (throughout its distributional range) to the environmental conditions experienced by the species. This allows us to explore to what degree responses vary and whether neglecting the existence of within species variation may affect the overall performance, predictive capacity and model transferability, and hence the conclusions derived from, Species Distribution Models. We also address the mechanisms potentially related to the ecological similarities or differences between deeply geographical-structured and divergent intraspecific lineages, namely: niche conservatism and niche divergence.

•GENERAL

METHODS•

GENERAL METHODS

On the suitability of the model species

Amphibians are an interesting group for studying biogeographical, ecological and evolutionary questions as they exhibit an immense variation between species in morphological features, life-history traits, reproductive strategies, habitat preferences and distribution, among others (Trochet et al., 2014; Marshall et al., 2018). They also show a marked within-species variation in phenotypic and life-history traits (e.g., Miaud, Guyetant & Faber, 2000; Gomez-Mestre & Tejedo, 2003; Laugen, Laurila, Räsänen & Merilä, 2003; Marangoni, Tejedo & Gomez-Mestre, 2008; Luquet, Lena, Miaud & Plenet, 2015; Liao, Luo, Lou, Lu & Jehle, 2016) and genetic differentiation levels and diversity (e.g., Alexandrino, Froufe, Arntzen & Ferrand, 2000; Zamudio & Wieczorek, 2007; García-París, Martínez-Solano & Velo-Antón, 2008; Milá, Carranza, Guillaume & Clobert, 2010; Teixeira, Gonçalves, Ferrand, García-París & Recuero, 2018). This intraspecific phenotypic and genetic variation has evolved in response to local conditions of the habitat where each species lives (including latitude, altitude, geology, climate, resource availability and population density) due to their high phylopatry and relatively limited mobility. Amphibians are one of the vertebrate groups most vulnerable to the current environmental change, involving habitat loss and fragmentation, emerging diseases, pollution, exotic species and climate change (Blaustein & Kiesecker, 2002; Beebee & Griffiths, 2005; Araújo, Thuiller & Pearson, 2006). In fact, they are the most rapidly declining taxonomic group (Stuart et al., 2004), with an extinction rate 200 times higher than the background extinction rate for the last 350 million years (Pimm, Russell, Gittleman & Brooks, 1995; Collins & Crump, 2009), and include more than 2092 threatened species, representing 40% of 6722 evaluated species of 7926 estimated existing species (IUCN, 2018). Amphibians are usually considered as good indicators of ecosystem health or habitat quality, earned their appellation of “canaries in a coal mine”. Nevertheless, recent studies suggest that amphibians may not be such good indicators as previously thought, as their populations may disappear without any early warning (Collins & Crump, 2009; Kerby, Richards-Hrdlicka, Storfer & Skelly, 2010).

We selected the Iberian newt, *Lissotriton boscai*, as a model species based on the following facts: (**a**) Previous phylogenetic and phylogeographic studies using nuclear and mitochondrial markers revealed that the species exhibits a marked phylogenetic and phylogeographic variation among populations, resulting from a "refugia-within-refugia" scenario of diversification in a Southern refugia during cold periods in the Plio-Pleistocene. This scenario promoted the emergence of two intraspecific lineages in allopatric local refugia with independent evolutionary histories (Martínez-Solano, Teixeira, Buckley & García-París, 2006; Teixeira et al., 2015); (**b**) The "refugia-within-refugia" scenario is in agreement with the hypothesis of historical climate stability. This hypothesis poses that refugia were characterized by stable ecological conditions during the Quaternary colder periods in the Iberian Peninsula (Svenning & Abellán, 2014). Therefore, *L. boscai* distribution is expected to be closer to equilibrium with contemporary climate (Svenning & Skov, 2004); (**c**) *L. boscai* is a very philopatric species that returns to its native habitat during the annual aquatic breeding phase (Rodríguez-García & Diego-Rasilla, 2006), restricting the short-term gene flow between populations; (**d**) The species inhabits closed water habitats (natural and artificial ponds, traditional freshwater troughs for cattle and human watering and water springs) or isolated shallow streams and rivers that allow an accurate record of the number of individuals per sampling site (e.g., Lizana, Ciudad & Pérez-Mellado, 1989; Garcia-Gonzalez & Garcia-Vazquez, 2011; Vries & Marco, 2017); (**e**) It is one of the Iberian amphibian urodele species best known for its ecology, with studies providing background information related to intraspecific and interspecific interactions (Díaz-Paniagua, 1979; Aragón, 2009a, b, 2011), reproductive biology (Brea, Galán, Ferreiro & Serantes, 2007) and the impact of pollutants on behaviour and development (Ortiz-Santaliestra, Marco, Fernández-Benéitez & Lizana, 2009; Ortiz-Santaliestra, Marco & Lizana, 2011); (**f**) Previous studies on geographical body size variation where restricted to Portugal or Spain, therefore not including populations from the whole distributional range of the species and failing to encompass the complete diversity of environmental, climatic and genetic features (Caetano & LeClair, 1999; Díaz-Paniagua & Mateo, 1999).

Model Species

General description, distribution and conservation status

The Iberian newt (*Lissotriton boscai*[1], Lataste, *1879*) is a small-bodied newt that belongs to the family Salamandridae (Amphibia, Caudata). Until recently, the species was included within the genus *Triturus*, but different studies revealed that this genus is paraphyletic, with a monophyletic assemblage of small-bodied species deserving their own genus under the *Lissotriton* designation including the following species: *L. boscai*, *L. helveticus*, *L. italicus*, *L. montandoni* and *L. vulgaris* (García-París, Montori & Herrero, 2004; Weisrock et al., 2006; Steinfartz, Vicario, Arntzen & Caccone, 2007). Its distribution is restricted to the western half of the Iberian Peninsula, with an altitudinal range reaching up to about 1870 m above sea level, and bounded by the Guadalquivir River to the south-east, by Sierra Morena and Sierra de Guadarrama in Central Spain, and by Picos de Europa to the north. This Iberian endemism is commonly found throughout Portugal and is widely distributed in Spain within the main mountain ranges encompassing the Sistema Central (e.g., Sierra de Francia, Sierra de Gata, Sierra de Gredos) as well as in Asturias and Galicia. Relatively well conserved populations can be also found towards its southern distribution range in Sevilla and Huelva. Its population densities decrease towards the limit of the species distribution in Sierra de Guadarrama and Sierra Morena (Pleguezuelos, Márquez & Lizana, 2002; Loureiro, Ferrand de Almeida, Carretero & Paulo, 2008).

An early study assessing the genetic intraspecifc variability throughout the distribution range of the species found chromosomal variation in 14 sampled

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[1] **Nomenclatural note**: Recently, Dubois and Raffaëlli (2009) proposed to raise to species rank the central and south-western Portuguese populations of *Lissotriton boscai* using the designation of *Lissotriton maltzani* (Boettger, 1879). These authors based their decision on the results provided by Martínez-Solano et al. (2006) regarding the existence of two different evolutionarily lineages in *L. boscai*. However, Martínez-Solano et al. (2006) merely suggested the possibility that both lineages were considered as two different species. In fact, Martínez-Solano et al. (2006) and Teixeira et al. (2015) suggested considering the two lineages as independent evolutionary significant units (ESUs, Moritz, 1994) for management and conservation efforts rather than as potential cryptic species. Moreover, the authors emphasized the need to perform further and wider behavioural, ecological, morphological and genetic studies at the population level, with a focus on the populations in south-western and central Portugal. The existing discrepancies and the lack of new evidence supporting the split of *L. boscai* into two different entities has resulted in the species taxonomic status remaining unchanged (Speybroeck, Beukema & Crochet, 2010). Therefore, the denomination of *L. maltzani* has not been accepted by the standing taxonomic committee of the Asociación Herpetológica Española (AHE) in its latest update of the "Lista patrón de anfibios y reptiles de España" (Carretero, Martínez-Solano, Ayllón & Llorente, 2018).

populations according to the C-heterochromatin band distribution and the DNA content (Herrero, 1991). Based on these karyological differences, the authors concluded that *L. boscai* populations belong to two different groups: the first one including the populations from Valle del Tietar (La Vera, Sierra de Gredos) and the second one including the remaining populations within the geographical distribution of the species. More recently, phylogenetic and phylogeographic analyses using two mitochondrial markers (mitochondrial gene coding for subunit 4 of the NADH dehydrogenase complex I [nad4] and the control region) and one molecular marker (β-fibrinogen intrón 7) revealed that populations belong to two well geographically–structured and highly divergent intraspecific lineages (lineage A and B), with relatively high levels of genetic distance, between 6.05-7.47% (Martínez-Solano et al., 2006; Teixeira et al., 2015). Lineage A is widely distributed throughout western Spain and most of Portugal, whereas lineage B is restricted to central and south-western Portugal (Fig. 4). The initial split between the two-mitochondrial lineages potentially took place during the Miocene, between 6-13 MYA (average 9 MYA). Then, they probably evolved in multiple allopatric refugia following the "refugia-within-refugia" pattern for southern European peninsulas during the Plio-Pleistocene (2.5-1.2 MYA), resulting in four different clades. The geographical distribution of the clades is mostly allopatric but overlap in some populations via secondary contact zones for mitochondrial and nuclear haplogroups (Martínez-Solano et al., 2006; Teixeira et al., 2015). Finally, the increasing advance in next-generation sequencing (NGS) technologies has allowed the development of microsatellite markers, some of them exclusive either to the lineage A or the lineage B, and de novo transcriptome assemblies and single nucleotide polymorphisms (SNPs) for the two lineages (Sequeira, Silva-Ferreira & Lopes, 2012; Nourisson, Muñoz-Merida, Carneiro & Sequeira, 2017).

Regarding the conservation status, *L. boscai* is listed as a Least Concern (LC) species based on The International Union for Conservation of Nature (IUCN) Red List of Threatened Species and the "Atlas y Libro Rojo de los Anfibios y Reptiles de España" (Pleguezuelos et al., 2002; Arntzen et al., 2009). The Iberian newt is legally protected by the Habitats Directive 92/43/EEC (Annex IV), the Bern Convention (Appendix III) and the Spanish normative (RD 1997/95). The major threats for the conservation of Bosca's newt include habitat loss and fragmentation, emerging diseases, pollution, introduction of exotic species and climate change—all of them responsible for the worldwide decline of amphibians (Collins & Crump, 2009). Recent studies have described two *common midwife toad virus* (CMTV)-like ranavirus affecting the species: the *Bosca's newt virus* (BNV) in Galicia and Picos de Europa National Park and the *Portuguese newt and toad ranavirus* (PNTR) (Price et al., 2014; Rosa et al., 2017). The latter is related to a mass mortality event in two populations from Serra da Estrella Natural Park, following an outbreak of a ranavirosis in 2011 and 2012. Individuals in Serra da Estrella Natural Park were also co-infected with *Batrachochytrium dendrobatidis* (Rosa et al., 2017). Agricultural intensification coupled with changes in land use and increased spraying of fertilizers have led to the loss and/or disturbance of preferred aquatic breeding habitats (Ferreira & Beja, 2013) and deleterious effects at different phases of *L. boscai* life cycle (Ortiz-Santaliestra, Marco & Lizana, 2005; Ortiz-Santaliestra et al., 2009; Ortiz-Santaliestra et al., 2011).

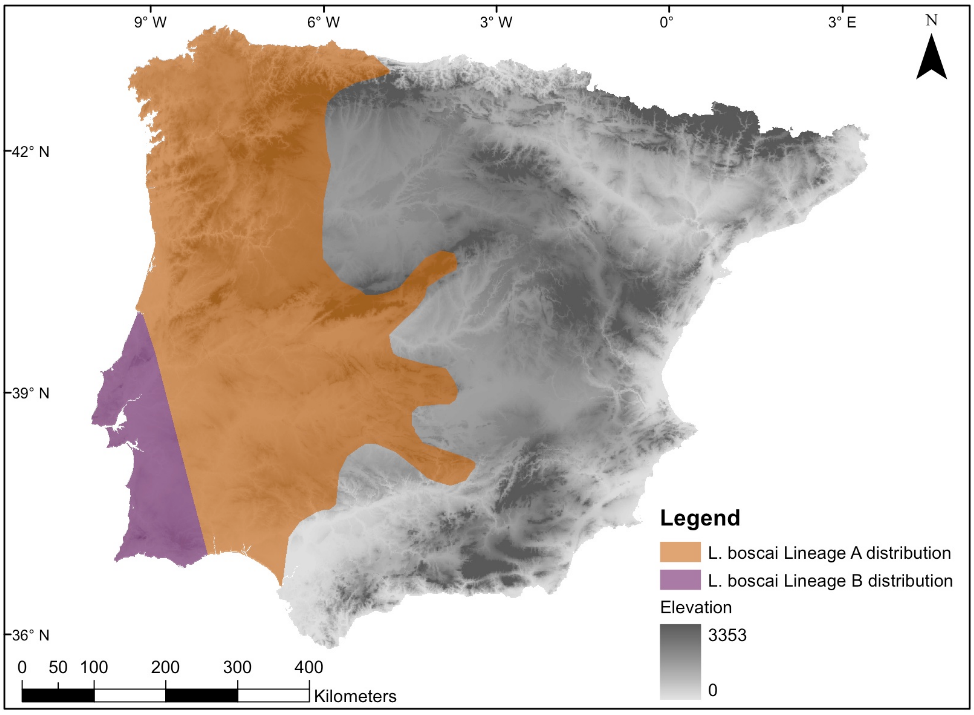

Figure 4 Distribution of *Lissotriton boscai*. Distribution range for lineage A is coloured in orange and distribution range for lineage B is coloured in purple —modified from Martínez-Solano et al. (2006). The background map shows the elevation, where darker grey represents higher altitude.

The introduction of the red swamp crayfish (*Procambarus clarkii*) in early 1970's has negatively impacted the distribution of the species in breeding sites due to a low embryo and larval survival in the presence of this predator (Cruz & Rebelo, 2005; Cruz, Rebelo

& Crespo, 2006). Forecasted climate change scenarios for the twenty first century reveal a mild impact on the potential distribution of the species. These models predict a contraction between 41% and 46% of the current potential species distribution for the period 2041-2070, and the match level between potential and observed distributions is predicted to shrink between 39% and 43% for the period 2041-2070 (Araújo, Guilhaumon, Neto, Pozo & Calmaestra, 2011).

Habitat, ecology and lifestyle

L. boscai inhabits a wide array of aquatic environments including shallow streams and rivers, temporal and permanent natural and artificial ponds, traditional freshwater troughs for cattle, water springs and, at some instances, reservoir tail waters (Fig. 5). These aquatic habitats span the two major climatic areas within the Iberian Peninsula: the drier Mediterranean domain and the most humid Atlantic domain, including transition zones between the two domains (Lizana et al., 1989; Garcia-Gonzalez & Garcia-Vazquez, 2011; Gómez-Rodríguez, Bustamante, Díaz-Paniagua & Guisan, 2012; Ferreira & Beja, 2013; Vries & Marco, 2017).

Figure 5 Habitat of *Lissotriton boscai*. (**a**) Cattle watering trough in Linares de Riofrío (Salamanca, Castilla y León). (**b**) Freshwater spring for human consumption in Villacondide (Asturias, Principado de Asturias). (**c**) Artificial pond in Monforte de la Sierra (Salamanca, Castilla y León). (**d**) Shallow river in Abelleira (La Coruña, Galicia). Photographs: Miguel Peñalver-Alcázar.

Adult newts in the aquatic phase are mainly active during daylight and twilight (Díaz-Paniagua, 1988; Lizana, Perez-Mellado & Ciudad, 1990). Individuals can be found in the aquatic habitat all year round. However, the species shows different interpopulational and sex-specific patterns of annual activity. For instance, newts from populations in southern Spain and central Portugal leave the aquatic habitat and enter in terrestrial dormancy (aestivation) during the summer. Nevertheless, newt may hibernate or spend the whole year in the water in northern and central populations (Lizana et al., 1989; Caetano & LeClair, 1999; Díaz-Paniagua & Mateo, 1999). Breeding activity may take place from October-January to June-August. *L. boscai* oviposition behaviour is similar to other newts such as *Triturus pygmaeus*, scattering their eggs both in space and time. *L. boscai* females do not lay their eggs in one clutch but individually wrapping these eggs in leaves, or adhering them to the underside of rocks (Díaz-Paniagua, 1986, 1989; Orizaola & Brana, 2003). This behaviour leads to a long oviposition period that may last from three or four months (January to March-April) up to the whole aquatic phase (Díaz-Paniagua, 1986; Lizana et al., 1989). Female size and number of eggs are not related (Brea et al., 2007). The youngest breeding individuals detected in the aquatic habitats are usually older than three years, but in some population males as young as two years old can also be found (Caetano & LeClair, 1999; Díaz-Paniagua & Mateo, 1999). Although females are larger than males (Fig. 6), *L. boscai* does not show a very marked sexual dimorphism compared to other newts (Caetano, 1982). Previous studies reported geographical body size differences between populations in Portugal and Spain. However, these studies are based on a small population sample size (six populations at best) and limited to either one of the two countries encompassing the species distribution (Caetano & LeClair, 1999; Díaz-Paniagua & Mateo, 1999).

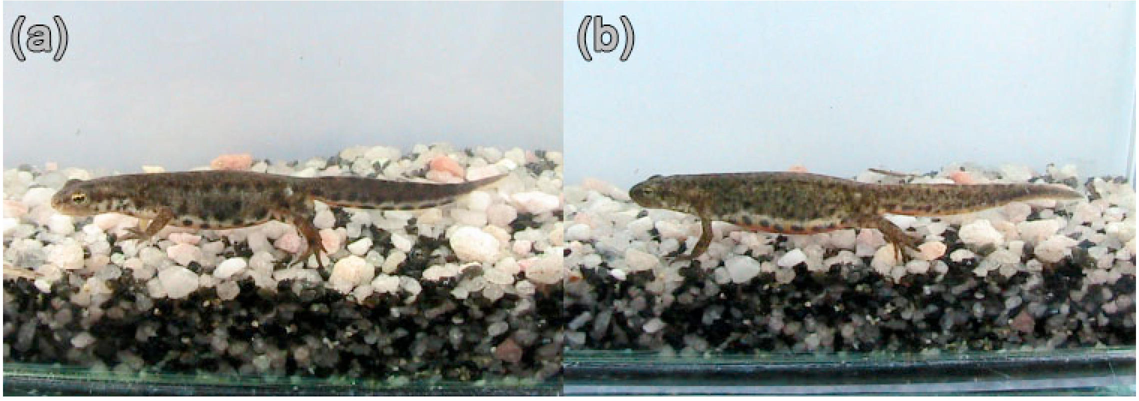

Figure 6 *Lissotriton boscai* adult (**a**) male and (**b**) female. Photographs: Pedro Aragón.

L. boscai males and females are not territorial (Aragón, 2009b), similar to other small-bodied newt species of the genus *Lissotriton* (Trochet et al., 2014). Male-to-male competition occurs mainly during courtship, with male(s) interfering in the courtship of other male(s) and displaying agonistic behaviours such as chases, pushes and bites (Faria, 1993, 1995; Aragón, 2009b). During foraging activities, males use trial-error tactics to detect food (regardless of the feeding activities of conspecific males), resulting in a lower degree of direct intraspecific competition. In contrast, females use social information to obtain food resources at closer distances and therefore are more prone to engage in agonistic interactions during feeding activities. These intraspecific interactions consist in single quick bites and never endured or resulted in injuries in experimental conditions (Aragón, 2009a). Interspecific interactions (competition and predation) have been described in several species of newts with which the species usually shares the aquatic habitat, such as *T. marmoratus* and *T. pygmaeus*, and ophidians including *Natrix natrix*, *N. maura* and *Vipera seoanei* (Díaz-Paniagua, 1979; Galán-Regalado, 1988; Lizana et al., 1990; Ayres, 2007).

Sampling methods

Newt morphometric measurements, genetic samples and relative density

Field samplings were performed during the early spring and summer of 2013 and 2015. Overall, 27 populations were sampled. Seventeen of those populations were sampled over two years (2013 and 2015), five only in 2013 and five only in 2015. The sampled populations represent both the geographic range and evolutionary history of the species.

L. boscai individuals were sampled by sweeping small and medium, small-mesh dip nets through the water and then transferred to plastic buckets filled with water from the same sampled habitat. Adult newts were sexed and measured *in situ* for body size (length from the snout to the posterior margin of the cloaca slit, snout-vent length, SVL) to the nearest millimeter. A small portion of the tail tip from 2 to 4 individuals was also collected for the genetic analysis (only in 2013). Genetic samples were preserved in 1 ml tubes with 100% absolute ethanol and stored in a portable deep-freezer at -20 ºC. The sampling effort in each population was recorded to calculate capture rate as a proxy of relative female and male density. Once all measurements were performed, adult newts were released into their native habitat. Any sampled juvenile newts were also released after capture. All sampling material was sterilized *ex situ* with 70% ethanol in water to avoid spreading diseases and pathogens.

Climatic and ecological variables at micro and macroscale

Several climatic and ecological variables describing the habitat of the Iberian newt at different scales were recorded for each sampled population. This methodology was common for **Chapters I and II**.

Ecological variables at the microscale were used to describe the physical-chemical characteristics and primary production of sampled populations. Nitrate concentration was measured using a HI 96728 nitrate portable photometer (Hanna Instruments Co., Spain). Conductivity, pH and water temperature were registered using a PC 5000H portable multi-parametric device coupled with phenomenal CO11 and 100 probes (VWR pHenomenal Instruments). To quantify primary production, two 250 ml water samples were also taken, preserved in a portable deep-freezer at -20 ºC and transferred to the Laboratorio de Cromatografía from the Museo Nacional de Ciencias Naturales (MNCN) to measure the concentration of chlorophyll a. The quantitative determination of chlorophyll a was performed using an ultraviolet/visible spectrophotometry. This technique is used to measure absorbance at the wavelengths corresponding to the chlorophylls maximum absorption after extraction with 90% acetate, and the resulting analytes are quantified by their molar extinction coefficient.

Ecological variables at the macroscale provided information on temperature, precipitation, evapotranspiration, primary production and topography of sampled populations. Temperature and precipitation variables were derived at 1-km resolution from the WorldClim 1.4 database (Hijmans, Cameron, Parra, Jones & Jarvis, 2005). The annual potential evapotranspiration was calculated following Thornthwaite (1948). Year-average monthly maps of Enhanced Vegetation Index (EVI) generated from the Moderate-Resolution Imaging Spectroradiometer (MODIS) payload sensor images (https://search.earthdata.nasa.gov/search) at a 1-km resolution were used to calculate mean annual primary productivity and primary productivity seasonality (Running et al., 2004). Finally, predator and competitor richness variables were derived from the Base de Datos Herpetológica of the Asociación Herpetológica Española (www.herpetologica.com) and the Atlas of Amphibians and Reptiles of Europe (http://na2re.ismai.pt/) of the Societas Europaea Herpetologica (http://www.seh-herpetology.org/) at 1-km and 10-km resolution, respectively. Climatic, ecologic and physiographic variables were managed, processed and calculated using Geographic information system (GIS) tools: ARCGIS 9.3 (ESRI, 2009) and IDRISI SELVA (Eastman, 2012).

Genetic, phylogenetic and phylogeographic analyses

The objective of these analyses was to verify that sampled populations were representative of previously described species phylogeographic structure in Martínez-Solano et al. (2006), and to calculate phylogenetic variables describing the phenotypic variability, evolutionary history and existing phylogenetic relationships among populations. For this, one mitochondrial DNA gene (mtDNA gene coding for subunit 4 of the NADH dehydrogenase complex I [nad4]) and seven polymorphic microsatellite loci were used. DNA was extracted from the tail-tips of adult newts and amplified, sequenced and genotyped following the protocols described in Martínez-Solano et al. (2006) and Sequeira et al. (2012), respectively. This methodology was common for **Chapters I and II.**

Mitochondrial DNA sequencing, phylogenetic and phylogeographic analyses

The mitochondrial DNA sequences were checked and edited using SEQUENCHER software (Gene Codes Corp., USA) and then visually aligned in MESQUITE 3.0.4 (Maddison & Maddison, 2015). Next, mtDNA sequences were reduced to haplotypes using FABOX 1.41 (http://users-birc.au.dk/biopv/php/fabox/) and the best-fit nucleotide substitution model was calculated based on the Bayesian information criterion (BIC) with JMODELTEST 2 (Guindon & Gascuel, 2003; Darriba, Taboada, Doallo & Posada, 2012). Phylogenetic analyses based on Bayesian inference were performed in BEAST 1.7 (Drummond, Suchard, Xie & Rambaut, 2012), specifying the optimal model of nucleotide substitution selected by JMODELTEST 2. Pairwise genetic distance matrix at the population level for the mitochondrial marker was calculated in MEGA 5.0 (Tamura et al., 2011). Newly generated mtDNA sequences were deposited in GenBank.

Microsatellite sequencing and population genetic diversity and differentiation

Regarding the seven polymorphic microsatellite loci, four of the seven loci used (Ltb4, Ltb10, Ltb11 and Ltb12) were previously described in Sequeira et al. (2012), and the remaining three (Ltb31, Ltb37 and Ltb25) were specifically developed for this thesis. Fragments were scored using GENEMAPPER 3.7 (Applied Biosystems). For all the microsatellite markers, standard genetic diversity indices were calculated with GENALEX 6.5 (Peakall & Smouse, 2006, 2012). Linkage disequilibrium (LD) and departures from Hardy–Weinberg equilibrium (HWE) were tested amongst the loci and populations with GENEPOP 4.2 (Raymond & Rousset, 1995; Rousset, 2008). Finally, the genetic differentiation between all population pairs was estimated using pairwise F_{ST} values (Weir & Cockerham, 1984) with GenoDive 2.0.b (Meirmans & Van Tienderen, 2004). Newly developed microsatellite sequences were deposited in GenBank.

Phylogenetic factor extraction

We included (phylo)genetic factors (PVs) as new predictors in statistical models to take into account both phylogenetic inertia and relationships among populations. The main aim was to include those PVs that might contribute to the explanation of body size and sexual size dimorphism rather that include those PVs that account for the largest part of the spatial (phylogenetic) variation (Diniz-Filho, de Sant'Ana & Bini, 1998; Diniz Filho, Rangel, Santos & Mauricio Bini, 2012; Diniz-Filho et al., 2012).

(Phylo)genetic factors were extracted from truncated pairwise genetic distance matrices for mitochondrial and microsatellite markers for the populations using a principal coordinate analysis (PCoA) following Diniz-Filho et al. (1998) and Aragón and Fitze (2014). The PCoA provided a set of mitochondrial and microsatellite PVs, which reflect either evolutionary relationships (mtDNA distance matrix) or gene flow among spatially close populations (F_{ST} matrix). The selection of the relevant PVs to enter in the statistical models was based on the significance of linear regressions between PVs and the residuals of the ecological models (Diniz-Filho et al., 2012). PCoA analyses were performed in R 3.0.2 (R Core Team, 2013), using the function “pcoa” from “ape 3.0-8” package (Paradis, Claude & Schliep, 2004).

General statistical analyses

Once the independent eco-environmental and phylogenetic factors were obtained and selected, these were used to construct ecological, phylogenetic and combined models (including both types of factors) by means of the Partial Least Squares Regression (PLSR) technique (Garthwaite, 1994; Wold, 1994; Carrascal, Galván & Gordo, 2009). PLSR models were fitted with the orthogonal scores algorithm (NIPALS) using a cross-validation procedure. The presence of spatial autocorrelation —the absence of independence between two sets of observations at a given spatial distance (Legendre, 1993; Borcard, Legendre, Avois-Jacquet & Tuomisto, 2004)— can impact the results of statistical models by biasing parameter estimates and/or overestimating the contribution of predictors (Legendre et al., 2002). Therefore, the potential existence of spatial autocorrelation was explored in PLSR models by calculating Moran's *I* and spatial correlograms based on the residuals of PLSR models including eco-environmental and phylogenetic factors (Diniz-Filho & Bini, 2005; Griffith & Peres-Neto, 2006).

The results provided by PLSR models were then corroborated using multiple regression models —generalised linear models (GLM) or linear mixed models (LMM)— following a model selection approach based on Akaike information criterion (AIC; Burnham & Anderson, 2002, 2004; Galván & Rey Benayas, 2011). All possible models were fitted and their second-order AIC (AICc) and AIC weight (AICw) scores were calculated. Models with a $\Delta\text{AICc} < 2$ were retained, as they have substantial support and are considered good models, and the relative variable importance of each predictor was calculated as the sum of the AICw of all selected models (Burnham & Anderson, 2002, 2004). All predictors were standardized before model building (Quinn & Keough, 2002). The rationale underlying the use of the GLM or LMM in addition to the PLSR approach was to assess the similarities between both methodologies. This type of comparison is appropriate because so far few studies on geographical body size and sexual size dimorphism variation have used the PLSR technique, whereas the model selection approach based on the AIC criterion is more common (e.g., Ficetola et al., 2010; Romano & Ficetola, 2010). This comparison was therefore useful to set a link with previous studies. Moreover, if results provided by both methodologies converge, it's reasonable to expect that the findings are robust. PLSR along with GLM was used in **Chapter I**, while PLSR along with LMM was used in **Chapter II**. PLSR and model selection analyses were performed in R 3.0.2 (R Development Core Team, 2012), using "pls 2.4-3" (Mevik & Wehrens, 2007) and "MuMIn 1.9.11" packages (Bartón, 2013), and spatial autocorrelation analyses were performed in SAM 4.0 (Rangel, Diniz-Filho & Bini, 2010).

Species distribution models

Species distribution models (SDMs) were used to estimate and characterize the potential distribution and niche of the Iberian newt, by modelling either the species as a whole or its two main intraspecific phylogenetic lineages. Then, the species and/or lineage level based-distribution models were used to predict the distribution of each other lineage, assessing the potential existence of intraspecific niche similarities or differences. Additionally, an environmental based-ordination technique approach was used to test the potential existence of niche equivalency and/or niche similarity between the species intraspecific phylogenetic lineages.

Distribution models and evaluation

Distribution models were built using nine pre-selected environmental variables and georeferenced occurrence records from previous phylogeographic studies on the Iberian newt (Martínez-Solano et al., 2006; Sequeira et al., 2012; Teixeira et al., 2015) and the field populations sampled during 2013 and 2015 (**Chapters I and II**), in which their geographic coordinates were recorded using a Garmin eTrex GPS device. Overall, 79 populations of *L. boscai* were used, including 66 populations from lineage A and 13 populations from lineage B. Distribution models were fitted using three different presence-only or presence-background algorithms:

- Bioclim was one of the first SDMs developed by scientist to map plant species distributions in Australia and has been widely used in the field since 1984 (Franklin, 2010; Booth, 2018). It is an environmental envelope-approach that uses presence-only data with no need of any background data or pseudo-absence data. Bioclim defines species potential range and ecological niche as the multi-dimensional rectilinear envelope that encloses all the species records (or they can be sorted out in different percentiles to include, for example, 95% or 90% of the occurrence localities) in the environmental space defined by the selected variables. The minimum and maximum records of the species along each selected environmental variable are used to define the rectilinear envelope in the environmental space (Fig. 7a). Therefore, Bioclim defines a minimal rectilinear envelope that assumes a binary relationship (suitable or unsuitable habitat) between the presence of a species and any environmental variable (Franklin, 2010).

- Ecological Niche Factor Analysis (ENFA) is a multivariate distance-based approach proposed by Hirzel, Hausser, Chessel and Perrin (2002). ENFA uses species occurrence locations and background data to calculate species habitat suitability, so it is regarded as a presence-background method. ENFA estimates species habitat suitability by computing uncorrelated factors —similar to Principal Component Analysis (PCA)— from several potential correlated environmental predictors that describe the multidimensional environmental space (i.e. the ecological niche) of a species. The analysis uses the niche concepts of species marginality and specialization. Marginality, the first factor extracted, describes the difference between the species mean and the global mean in the multidimensional environmental space (Fig. 7b). Marginality values vary from 0 to 1, and therefore it informs whether a species occurs in widespread conditions through the study area (low values) or whether a species occurs in conditions that are rare (high values). Specialization, the following factors extracted, describes the ratio of variance between the species distribution in the environmental space and the global environment distribution (Fig. 7b). Specialization informs about how restricted the species niche is in relation to the study area; higher values indicate that species occurs in a narrow range of conditions (Hirzel et al., 2002).

- MaxEnt is a machine-learning modelling approach that uses both species presence data and a randomly selected background of pseudo absences, with their corresponding environmental features (Phillips, Anderson & Schapire, 2006). MaxEnt combines statistical and Bayesian inference to estimate the multivariate probability distribution of habitat suitability conditions following the principle of maximum entropy. This principle states that the probability distribution that best represents an unknown occurrence data distribution is the one with the maximum entropy —the one that is more uniform or closest to uniform— subject to known constraints imposed by the information related to the observed occurrences and the environmental conditions of the study area. Therefore, MaxEnt estimates distributions that should be in agreement with everything that is known (even if it is incomplete and not assuming anything about the unknown) from the information inferred upon the environmental conditions of the occurrence locations (Phillips et al., 2006; Elith et al., 2011).

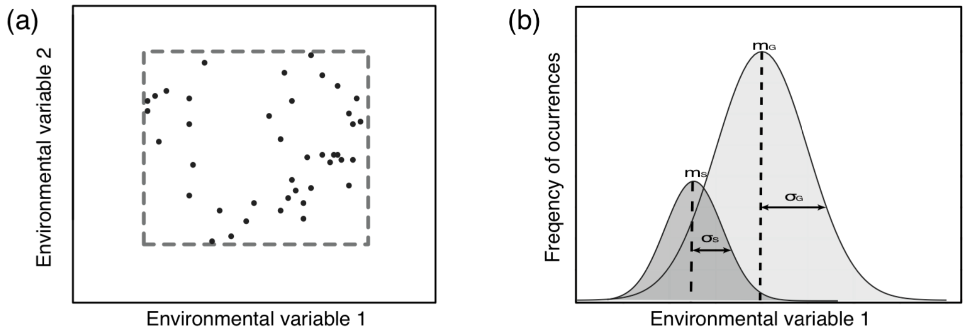

Figure 7 Conceptual representation of the different approaches for Species Distribution Models: (**a**) Bioclim: the dashed rectangle represents the minimal rectilinear envelope that encloses the minimum and maximum values of the environmental variables for all occurrences (dots). (**b**) Ecological Niche Factor Analysis (ENFA): the distribution of the focal species for any given environmental variable (dark grey) may differ from the global distribution for the study area (light grey bars). This difference may be regarding to the mean ($m_S \pm m_G$; marginality) and/or to the standard deviations ($\sigma_S \pm \sigma_G$; specialization). Figure 7b is adapted from Hirzel et al. (2002).

The accuracy of the different modelling techniques was evaluated using an intra and inter-model cross-evaluation approach. The accuracy assessment was measured using four different and common indices (Fielding & Bell, 1997; Manel, Williams & Ormerod, 2001; Boyce, Vernier, Nielsen & Schmiegelow, 2002; Hirzel, Le Lay, Helfer, Randin & Guisan, 2006): the true positive rate (TPR), the false negative rate (FNR), the area under the curve (AUC) from the receiver operating characteristic curve (ROC) and the continuous Boyce Index (CBI).

Niche overlap, similarity and equivalency

The potential statically significant differences between the lineage A and lineage B ecological niches was tested using the environmental-PCA method proposed by Broennimann et al. (2012) and implemented in the R package "ecospat" (Di Cola et al., 2017). The environmental-PCA method works in four steps: (**1**) building an environmental space for the study area, gridded with a resolution of 100 x 100 cells, extracting environmental values for both the occurrence records of the two lineages and random background points; (**2**) transforming the occurrences of the two lineages into density of occurrence by a kernel smoothed density function and plotting them along the gridded environmental-PCA space; (**3**) quantifying the observed niche overlap along the gradients of the environmental-PCA by calculating the Schoener's *D* index, which ranges from 0 (no overlap) to 1 (complete overlap; Warren, Glor & Turelli, 2008); (**4**) performing a statistical test for the niche similarity and niche equivalence hypotheses (Warren et al., 2008; Broennimann et al., 2012). The niche similarity test assesses whether the ecological niches of two lineages are more or less similar (niche conservatism *versus* niche divergence) than expected by chance, while the niche equivalence test assesses whether the ecological niche occupied by two lineages are identical.

Further specific information about methods and procedures are provided in **Chapters I to III** as well as in the **Appendices**.

•RESEARCH

CHAPTERS•

CHAPTER I

Sex-dependent implications of primary productivity and conspecific density on geographic body size variation in a newt: disentangling local, large scale and genetic factors

Peñalver-Alcázar, M., Martínez-Solano, I., Sequeira, F and Aragón, P. 2017. Journal of Biogeography 44: 2096-2108.

ABSTRACT

Aim

Geographic body size variation has interested biologists since the 19th century. However, there is no consensus regarding the mechanisms involved, especially in amphibians. At the intra-specific level, different approximations and hypotheses have addressed this problem, but no study has integrated information at different spatio-temporal ecological and (phylo)genetic scales while considering differences between sexes. We investigated the mechanisms involved in geographic body size variation in the Iberian newt (*Lissotriton boscai*), accounting for the main hypotheses potentially explaining variation in this phenotypic trait in amphibians.

Location

Iberian Peninsula.

Methods

We used a novel multivariate technique (partial least squares regression) that accounts for interdependence among variables while allowing for the inclusion of multiple local and macroscale predictors. We considered intra-specific genetic differentiation including information from the mitochondrial and nuclear genomes in populations representative of the species' distribution. We addressed causal hypotheses of heat conservation, temperature-related development, water and food availability, seasonality, competition and predation.

Results

Annual primary productivity and intra-specific competition were of greatest relevance to explain body size variation in females, while seasonality of primary productivity was more important in males. Differences between sexes are interpreted based on previous experiments on the behavioural ecology of this species.

Main conclusions

This study shows that sex-dependent implications of competition for food drive geographic body size variation in an urodele amphibian, supporting the density-resource hypothesis. Our results suggest that food availability, conspecific density and competition are important in explaining intra-specific body size variation in amphibians. This study highlights how selecting model species with an experimental background can help interpret geographic variation in phenotypic traits. We emphasize the value of simultaneously considering alternative hypotheses of phenotypic variation at different ecological and (phylo)genetic spatio-temporal scales in revealing potentially hidden relationships.

Keywords: Amphibians, body size variation, ectotherm, genetic variation, intra-specific competition, local scale, macroscale, partial least squares regression, primary productivity

INTRODUCTION

Body size is a functional trait strongly linked with species ecology and evolution (Roff, 1992) and its geographic variation has interested biologists since the seminal work of Karl Bergmann (Bergmann, 1847). Bergmann's rule states that body size increases at higher latitudes or altitudes (where temperatures are cooler), as an adaptive mechanism of heat conservation. It was originally proposed at the inter-specific level and extended later to the intra-specific level by Rensch (1938) and (Mayr, 1956). While several studies have shown support for Bergmann's rule (e.g., Meiri & Dayan, 2003), the generality of this pattern and its underlying mechanism(s) have been criticized in endotherms (e.g., McNab, 1971; Geist, 1987) and even more so in ectotherms. Particularly amphibians are considered a puzzling group (Ashton, 2002; Olalla-Tárraga & Rodríguez, 2007; Adams & Church, 2008). Alternative mechanistic hypotheses have been proposed to explain geographical body size variation, which consider different physiological and life history traits, including seasonality (Boyce, 1979), food availability (Meiri, Yom-Tov & Geffen, 2007; Huston & Wolverton, 2011) and competition (Desdevises, Legendre, Azouzi & Morand, 2003; Olson et al., 2009). Additional hypotheses are taxon-specific, such as desiccation tolerance in amphibians (Ashton, 2002 but see Gouveia & Correia, 2016 for a recent reformulation of this hypothesis).

Geographical body size variation studies face several challenges. First, environmental factors are often correlated and share spatial variation with genetic factors. Consequently, some authors have suggested performing phylogenetic and phylogeographic analyses, both at the inter-specific (Lomolino, Sax, Riddle & Brown, 2006) and intra-specific (Ashton, 2004) levels. Second, mechanistic hypotheses are not necessarily exclusive and may have synergistic or counteracting effects (Mayr, 1956; Lomolino et al., 2006). Third, the rapid increase in the availability of digitised macroscale environmental factors has led to a bias against local drivers of phenotypic diversification. Finally, the observational nature of these studies and the multiple non-exclusive hypotheses imply working with large multivariate datasets. Principal component analysis, classical multiple linear regressions, and less frequently, information-theoretic approaches, have been used alone or combined to deal with this (Ficetola et al., 2010; Aragón & Fitze, 2014). However, these techniques have several drawbacks, including non-independence among predictors, loss of degrees of freedom or failure to maximize the variance explained in the response variable (Legendre & Legendre, 1998). These limitations are addressed using partial least squares regression (PLSR), a promising technique recently introduced in ecological studies (Mevik & Wehrens, 2007; Carrascal, Galván & Gordo, 2009).

Here we describe range-wide patterns of body size variation in an urodele amphibian, the Iberian newt (*Lissotriton boscai*), and unravel the underlying mechanisms using an integrative, multiscale approach considering historical factors. We built models for each sex, using local and macroscale predictors while accounting for population genetic structure. We selected *L. boscai* because previous studies have reconstructed its evolutionary history (Martínez-Solano, Teixeira, Buckley & García-París, 2006; Teixeira et al., 2015), and background information exists on local factors potentially affecting body size, including water-chemistry characteristics (Ortiz-Santaliestra, Marco & Lizana, 2011) and intra-specific interactions (Aragón, 2009b). Moreover, in the context of the global amphibian declines (Collins & Crump, 2009), body size variation within species can reveal factors (natural *versus* anthropogenic) affecting population health. We sampled 23 populations representative of the species' environmental, phylogenetic and geographic variation. Body size and genetic samples were taken and the newts' environment was characterized at the micro and macroscale. We tested eight mechanistic hypotheses potentially involved in geographic body size variation: (1) Bergmann's rule or heat conservation: larger body size allows reducing heat loss in colder environments (Bergmann, 1847). According to Olalla-Tárraga and Rodríguez (2007), in amphibians this mechanism might rely on the species thermoregulatory behaviour; (2) Water availability: larger body size favours higher desiccation tolerance due to a small surface to volume ratio (Ashton, 2002); (3) Converse water availability: larger body size is related to higher availability of water resources (Ficetola et al., 2010); (4) Primary productivity: higher availability of food resources leads to an increase in body size (Huston & Wolverton, 2011); (5) Endurance: larger body size is related to higher body fat reserves in colder and highly seasonal locations, increasing survivorship during adverse conditions (Boyce, 1979; Ashton, 2002); (6) Seasonality: individuals living in populations with longer favourable activity seasons are larger than those living in populations with shorter and fluctuating activity seasons (Boyce, 1979); (7) Competition and/or predation: competition and predation cause species to deviate from their optimal body size (Damuth, 1993). Specifically, recent studies in amphibians suggest that a higher number of conspecific competitors can lead to a smaller body size (Green & Middleton, 2013) and that a higher number of inter-specific competitors and/or predators can result in smaller body size (Ousterhout, Anderson, Drake, Peterman & Semlitsch, 2015); (8) Temperature size rule: development and growth rates are lower in colder environments, leading to longer embryonic development and growth periods, which result in delayed maturity and larger adult body size (Angilletta & Dunham, 2003).

We used PLSR to assess the relationship and relative importance of environmental predictors related to these hypotheses in *L. boscai*, considering relevant (phylo)genetic predictors. This study entails important advances: 1) we applied a novel multivariate technique (PLSR) that allows including a large set of variables while accounting for their interdependence; 2) we used multiple local and macroscale predictors to encompass several mechanistic hypotheses; and 3) we accounted for intra-specific genetic structure using molecular markers in the mitochondrial and nuclear genomes. Mitochondrial DNA sequences reveal deeper historical processes whereas nuclear microsatellites inform about more recent population events (Freeland, Petersen & Kirk, 2011). To our knowledge this is the first study integrating local and macroscale abiotic and biotic factors for both sexes and accounting for deep and recent genetic structure, while explicitly dealing with multicollinearity and/or redundancy.

MATERIALS AND METHODS

Study species

The Iberian newt, *Lissotriton boscai* (Lataste 1879), is endemic to the western Iberian Peninsula, ranging from sea level to 1870 m a.s.l. (Fig. 8). Densities are greater in central and northern populations than in southern and easternmost ones. It inhabits diverse aquatic environments including shallow streams, rivers, natural ponds, cattle troughs and water springs (García-París, Montori & Herrero, 2004). Females are larger than males, and seasonal activity varies among populations and between sexes (Caetano & LeClair, 1999; Díaz-Paniagua & Mateo, 1999). At the beginning of the breeding season, males arrive to the aquatic habitat first, followed by a gradual arrival of females (Faria, 1995; Caetano & LeClair, 1999). Neither sex shows territorial behaviour. Females compete amongst themselves for food, resulting in agonistic interactions characterized by single quick and usually harmless bites. Males compete with other males during courtship, displaying agonistic behaviours including chases, pushes and bites (Faria, 1995; Aragón, 2009a, b). Inter-specific interactions with other newt species (*Triturus marmoratus* and *Triturus pygmaeus*) exist and known predators include reptiles (*Natrix natrix*, *Natrix maura* and *Vipera seoanei*) and amphibians (*T. marmoratus*, *Salamandra salamandra*; (García-París et al., 2004). Phylogeographic analyses revealed two major geographically–structured lineages, each containing three sub-clades (Martínez-Solano et al., 2006; Teixeira et al., 2015).

Data collection

From March to June 2013, we sampled 23 populations of the Iberian newt (Fig. 8). Adult newts were caught in the aquatic phase and measured *in situ* for body size (snout-to-vent length; SVL). Tail tips were collected for genetic analysis, preserved in 1 ml tubes with 100% ethanol and stored at -20 ºC. Sampling time was recorded to calculate capture rate as a proxy for the relative population density. We obtained SVL measures for 457 individuals (255 females and 202 males) and 87 genetic samples. Sampled populations included small watering troughs, small natural and artificial ponds, shallow streams and rivers. The populations' small dimensions allowed us to cover the whole habitat multiple times to capture as many newts as possible. Vegetated margins were also screened for newts. Samples were taken from mid-late morning to late evening, as adult newts in the aquatic phase are mainly active during daylight and twilight (García-París et al., 2004).

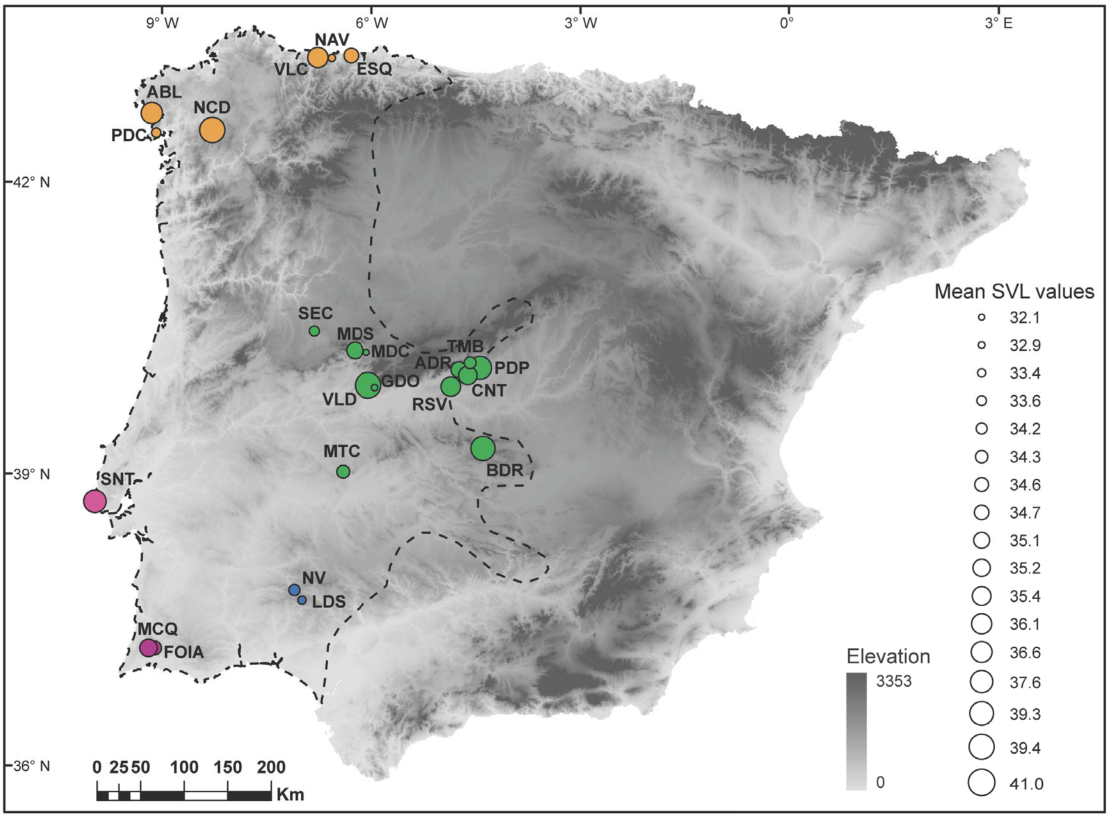

Figure 8 Distribution of the 23 sampled populations (coloured circles) within the geographical range of *Lissotriton boscai* (depicted by the dashed line) in the Iberian Peninsula. Background map shows the elevation, where higher intensity in the grey scale represents higher altitude. The width of the circles is proportional to mean snout-to-vent length (SVL) values and colours represent the different clades (Martínez-Solano et al. 2006; Fig. A1.2, Appendix 1.2). Population names correspond to population codes in Table A1.3, Appendix 1.3.

Microhabitat variables were measured at each population, including nitrate concentration (using a nitrate photometer), conductivity, pH and water temperature (using a multi-parametric device). We also took two 250 ml water samples to quantify chlorophyll a concentration, a proxy for primary productivity, kept them at -20 ºC in a portable freezer and transferred them to the laboratory for spectrophotometric analyses (American Public Health Association, 1989). These parameters are biologically relevant for amphibians and standard measures of water quality and conservation status (Dodd, 2010). Water temperature is related to embryonic development, metamorphosis and growth rates in amphibians (Morrison & Hero, 2003) and may affect and inform on activity of individuals in this species (Caetano & LeClair, 1999; Díaz-Paniagua & Mateo, 1999). Higher nitrogen concentration and acidification at breeding sites have been linked to global amphibian declines (Collins & Crump, 2009). In *L. boscai*, nitrogen excess and low pH cause higher mortality and delayed growth rates in embryos and larvae, and physiological and behavioural alterations in adults (Ortiz-Santaliestra et al., 2011). Once measurements and tissue samples were taken, individuals were released at their capture point.

We obtained temperature and precipitation variables with a resolution of 1-km from the WorldClim database (Hijmans, Cameron, Parra, Jones & Jarvis, 2005) for macroscale characterization of each population. We selected those variables related to our main hypotheses. Annual mean temperature was used to test Bergmann's and temperature-size rules; temperature and precipitation seasonality were selected to test the endurance and seasonality hypotheses; and annual precipitation was used to test the water and converse-water availability hypotheses. We calculated annual potential evapotranspiration following Thornthwaite (1948). We computed year-average monthly values of enhanced vegetation indexes (EVI) for 2012-2013 using satellite images at a 1-km resolution from MODIS (https://modis.gsfc.nasa.gov) as a proxy for primary productivity in sampling sites. We also calculated primary productivity seasonality as 100*(std. dev. of mean monthly EVI values / mean of the mean monthly EVI values). To account for potential effects of known predators and competitors of *L. boscai*, we derived predator and competitor richness from the atlases of the Asociación Herpetológica Española (www.herpetologica.com) and Societas Europaea Herpetologica (http://www.seh-herpetology.org/) at 1-km and 10-km resolution, respectively. Finally, we recorded the capture date, as the day number within the year, of each population in order to measure and control for temporal autocorrelation.

Genetic sequencing and phylogenetic factor extraction

Genetic analyses aimed at: 1) verifying that sampled populations were representative of previously described haplotype clades by comparing Bayesian phylogenies from our sampling with those in Martínez-Solano et al. (2006) (Supporting Information, Appendix 1.2), and 2) accounting for (phylo)genetic variation in final combined models by including relevant phylogenetic factors as predictors (Diniz-Filho, de Sant'Ana & Bini, 1998; Aragón & Fitze, 2014).

These analyses were performed with two different molecular markers: one mitochondrial DNA gene (*nad4*) and seven polymorphic microsatellite loci. We sequenced and genotyped samples using the protocols described in Martínez-Solano et al. (2006) and Sequeira, Silva-Ferreira and Lopes (2012), respectively (Appendices 1.2 and 1.3). We calculated a pairwise genetic distance matrix at the population level for the mitochondrial marker (Table A1.1, Appendix 1.2) in MEGA 5.0 using the Tamura-Nei model with gamma-distributed substitution rates among sites (Tamura et al., 2011). For microsatellite markers, we estimated standard genetic diversity indices (average sample size over loci, number of alleles, allelic richness, observed heterozygosity, and expected heterozygosity) and tested for linkage disequilibrium (LD) and departures from Hardy-Weinberg equilibrium (HWE) among the loci and populations (details in Appendix 1.3). We also estimated genetic differentiation between all populations pairs using pairwise F_{ST} values (Weir & Cockerham, 1984) and identified values significantly > 0 through 999 permutations (Table A1.4, Appendix 1.3) using GENODIVE 2.0.b (Meirmans & Van Tienderen, 2004). F_{ST} estimates the genetic differentiation among populations regarding their inherent genetic structure, which may express the degree of evolutionary independence of populations (Freeland et al., 2011). Then, to account for phylogenetic inertia and inter-population genetic variability in statistical models, pairwise genetic distance matrices for mitochondrial and microsatellite markers were used to derive independent (phylo)genetic eigenvectors (PVs), following Diniz-Filho et al. (1998) and Aragón and Fitze (2014). We performed principal coordinate analyses (PCoA) based on a double-centred pairwise genetic distances matrix among populations to extract independent PVs for mitochondrial and microsatellites distance matrices. Similarly to principal components analysis (PCA), PCoA yields orthogonal PVs, with coefficients that are informative of variation and relationships among samples in a reduced multivariate space of (phylo)genetic relatedness. PCoA provides a set of mitochondrial and microsatellite PVs. These (phylo)genetic predictors may reflect either evolutionary relationships at different temporal scales (mtDNA distance matrix) or some degree of gene flow among spatially close populations (pairwise F_{ST} matrix).

Ecological and combined model building

We first calculated the mean, minimum and maximum SVL for each population and sex. We then performed Pearson's correlations between pairs of body size variables (mean, minimum and maximum) to test the degree of collinearity between variables and assess the suitability of performing different analyses with each variable. We found a strong and significant correlation between the three pairs of variables for both sexes (Pearson's $r > 0.83$, $P < 0.0001$ in all cases), indicating they are likely to generate similar results. Therefore, we only used mean SVL in subsequent analyses. Mean SVL is commonly used in studies on geographical body size variation of urodele amphibians at the intra-specific level (e.g., Ficetola et al., 2010; Romano & Ficetola, 2010), and hence results are comparable with previous findings. We also performed Pearson's correlations between mean SVL of females and males and assessed the sexual dimorphism in body size by fitting a linear mixed model with mean SVL as dependent variable, sex as a fixed factor, and population as a random factor. Finally, we used mean SVL in PLSR to disentangle potential mechanisms involved in geographical body size variation. PLSR combines features of multiple linear regression and PCA and has been used in econometrics and chemometrics and more recently in ecological studies (Wold, Sjöström & Eriksson, 2001; Carrascal et al., 2009). PLSR overcomes limitations of classical multivariate techniques, providing robust results in situations with many interrelated predictors (Mevik & Wehrens, 2007; Carrascal et al., 2009). PLRS relies on the extraction of orthogonal latent-factors (PLSR components), which result from the linear combination of predictors and response variables. PLSR components are extracted iteratively and determined from the residual variation not explained by the preceding latent-factor. They account for a successively lower percentage of the original variance (explanatory capacity, R^2) of the response variable while maximising the information explained by the predictors. Most of the variance in the explanatory variables is concentrated in the first few PLSR components and less important PLSR components are discarded (e.g., via cross-validation), reducing the multidimensionality. The PLSR technique balances information in predictors and response variable, decreasing the potential effect of higher but irrelevant predictor variations. Thus, PLSR overcomes intercorrelation issues among variables by deriving orthogonal PLSR components as in PCA. However, PLSR has an advantage over PCA by including the response variable(s) in components calculation. PLSR components can be described using the predictor weights within each component, which indicate the direction and strength of the relationship between predictors and the dependent variable. The contribution of each predictor (i.e., explained information) within each component can be assessed through their squared weights (Wold et al., 2001; Carrascal et al., 2009) and the variable importance for projection (VIP). Predictors with VIP > 1 are the most relevant for explaining the dependent variable (Wold et al., 2001).

The PLSR model building was performed using mean SVL as the response variable and different sets of predictors. We followed this with two subsequent steps: 1) ecological models (only ecological predictors), and 2) combined models (ecological + PVs predictors from PCoA). Selecting and including the correct number of relevant PVs is a crucial step because too many or too few can lead to overestimation or underestimation, respectively (Desdevises et al., 2003; Diniz-Filho et al., 2012). Among the different existing approaches to deal with this issue, we followed the selection criterion based on the significance of linear regressions on the relationship between PVs and the residuals of the ecological models. This approach is better for selecting relevant PVs than using either the first few PVs (based on a broken-stick criterion) or the whole set of PVs derived from a PCoA (Desdevises et al., 2003; Diniz-Filho et al., 2012). Including only the first PVs with the highest eigenvalues (higher phylogenetic distances and thus ancient phylogenetic relationships) would neglect PVs with lower eigenvalues (lower phylogenetic distances and thus more recent phylogenetic relationships). However, if all PVs were included in the models, phylogenetic relationships would explain all variation in the studied trait, which would not be realistic for a single phenotypic trait. This method has been successfully applied at inter-specific (Diniz-Filho et al., 2012) and intra-specific levels (Aragón & Fitze, 2014).

PLSR models were performed separately for females and males due to the existence of sexual size dimorphism, and because each sex can be affected differently by ecological, social and evolutionary factors (Shine, 1989), as reported in this species (Aragón, 2009a, b, 2011). We fitted PLSR models with the orthogonal scores algorithm (NIPALS) and selected components that remained significant after a cross-validation procedure (default setting ‘CV’ in ‘pls’ package; Mevik & Wehrens, 2007), which divides the data set into 10 randomly selected subsets. The importance of PLSR components was interpreted using their R^2, considering predictors of greater relevance when VIP > 1 and explained information (i.e., square of predictor weights*100) > 10%

within at least one component. Interactions among predictors were not considered because it is not feasible to test them when the number of predictors is as high as in our study. Finally, we used the Akaike information criterion (AIC) (Burnham & Anderson, 2004) for model selection to corroborate the results of PLSR models (Galván & Rey Benayas, 2011), and provide results comparable to previous studies (e.g., Ficetola et al., 2010; Romano & Ficetola, 2010). We ran generalised linear models (GLM) with predictors with VIP > 1 and fitted all possible models, and calculated their second-order AIC (AICc) and weight AIC (AICw) scores. We selected models with a ΔAICc < 2 and calculated the relative variable importance of each predictor as the sum of the AICw of all selected models (Burnham & Anderson, 2004). Before model building, predictors were standardized (Quinn & Keough, 2002).

Finally, we explored the potential existence of spatial autocorrelation. Moran's *I* and spatial correlograms were calculated based on the residuals of the final combined models (Appendix 1.1). PLSR and model selection analyses were performed in R 3.0.2 (R Core Team, 2013), using 'pls 2.4-3' and 'MuMIn 1.9.11' (Bartón, 2013), and spatial autocorrelation analyses in SAM 4.0 (Rangel, Diniz-Filho & Bini, 2010).

RESULTS

General results

We found body size variation among the 457 individuals sampled across the 23 study populations (Fig. 8). Mean SVL ranged from 34.6 to 43.3 mm in females, and from 29 to 34.3 mm in males. While there is little overlap in the size distributions between sexes, in some populations the largest males were larger than the smallest females. Females were significantly larger than males across all populations ($F_{1,21} = 257.30$, $P < 0.0001$; mean ± SE, Females: 37.4 ± 0.40, N = 255; Males: 31.9 ± 0.40, N = 202). Variation in the sexual dimorphism index [(mean length of larger sex/mean length of smaller sex) – 1] among populations ranged from 0.10 to 0.26. The correlation between mean SVL of females and males was significant and positive (Pearson's $r > 0.76$, $P < 0.0001$).

Phylogenetic analyses were concordant with previous studies (Martínez-Solano et al., 2006; Teixeira et al., 2015), Fig. A1.2, Appendix 1.2), confirming that sampled populations are representative of intra-specific haplotype clades. Standard genetic diversity measures for microsatellite loci are shown in Table A1.3, Appendix 1.3. For all populations and across all loci, there were no significant deviations from Hardy-Weinberg or linkage equilibrium after Bonferroni correction (Table A1.3, Appendix 1.3). While most pairwise F_{ST} comparisons were significantly different from zero, some produced non-significant results, indicating gene flow between spatially close populations (Table 1.4, Appendix 1.3). Therefore, the incorporation of PVs derived from the pairwise F_{ST} matrix into combined models allowed to consider potential non-independence among several populations.

Final Combined Models

PCoA analyses provided 23 and 22 PVs for males and females, respectively. For female SVL, residuals of ecological models were significantly correlated with mitochondrial *nad4*-PVs 6 and 14, and F_{ST}-PVs 2, 4 and 22 (Pearson's $r > |0.43|$, $P < 0.05$ in all cases). For male SVL, residuals of ecological models were significantly correlated with the F_{ST}-PV11 (Pearson's $r = -0.45$, $P = 0.03$). Therefore, these PVs were included in the final combined models. This procedure was not trivial since three PVs (*nad4*-PV6, F_{ST}-PV2 and F_{ST}-PV11) had a VIP > 1 or explained information > 10% in the PLSR components (Tables 1 and 2). Moran's *I* correlograms showed that there was no spatial structure in the residuals of final combined models (Fig. A1.1, Appendix 1.1).

When accounting for genetic variation among populations in combined models of mean SVL, we found differences and similarities between males and females regarding the number of retained factors and the relationship and relevance of ecological predictors. The PLSR models provided three significant components explaining 89.6% of the original variance for females (PLRS-1: $r = 0.79$, $P < 0.0001$; PLRS-2: $r = 0.75$, $P < 0.0001$; PLRS-3: $r = 0.6$, $P < 0.05$; Table 1), and one significant component explaining 48.1% of the original variance for males (PLSR-1: $r = 0.706$, $P < 0.001$; Table 2). The relationship of most predictors had the same direction for both sexes in PLRS-1 (14 out of 17, Tables 1 and 2), verifying the robustness of our models. The three predictors with different directions were not considered relevant according to VIP < 0.7 in all cases) and information explained in PLRS-1 (< 2.5% in all cases).

Primary productivity-related variables were relevant for both sexes (according to VIPs and explained information in at least one PLRS component, and relative variable importance in AIC models), but sex-specific difference existed. Mean annual primary productivity at the macroscale was more relevant for females (mean EVI, Table 1; Fig. 9a, c), whereas its seasonal fluctuation was more relevant for males (EVI seasonality, Table 2; Fig. 9d). Furthermore, primary productivity at the microhabitat level (chlorophyll a concentration) was slightly more relevant for females (VIP = 1.48, explained information in PLSR-1 = 11.6%, Table 1) than for males (VIP = 1.18, explained information in PLSR-1 = 7.8%, Table 2). Similarly, annual mean temperature and potential evapotranspiration were more relevant in males than in females (Tables 1 and 2; Fig. 9), and this difference was stronger in potential evapotranspiration (females: VIP = 0.53, explained information in PLSR-1 = 7.8%; males: VIP = 1.72, explained information in PLSR-1 = 16.8%). Finally, a striking difference between sexes was that relative density was a highly relevant predictor of female SVL (VIP = 1.27, explained information in PLSR-1 = 10.24%; Fig. 9a), but not of male SVL (VIP = 0.18, explained information in PLSR-1 = 0.16%). Other predictors showed a moderate influence, since at least one of the selection criteria was not met (i.e., their VIP < 1 or explained information < 10% in PLRS, or the predictors were not selected by AIC models). These were temperature seasonality, conductivity and water temperature for females (Table 1), and chlorophyll a concentration, mean EVI, precipitation seasonality and annual precipitation for males (Table 2). The remaining predictors were less important in PLRS models for both sexes (i.e., both VIP < 1 and explained information < 10%).

Overall, individuals in populations with higher primary productivity and lower annual temperature, or smaller primary production fluctuations at the macroscale present larger body sizes. The ecological factor showing the strongest sex-bias was the local conspecific density, which was negatively associated with body size in both sexes. However, for females this predictor was among the three of greatest relevance, whereas for males it was among the three of lowest relevance (Tables 1 and 2).

Table 1 Predictor weights for selected components of partial least squares regression (wPLSR) of female mean snout-to-vent length (SVL) of Iberian newts and their variable importance for projection (VIP), and relative variable importance (Relative VarImportance) in Akaike information criterion models. R^2 is the proportion of the original variance of female mean SVL explained by each PLSR component. Bold lettering denotes predictors with explained information (square of predictor weights*100) > 10% or VIP > 1.

Predictor variable	VIP	wPLSR-1	wPLSR-2	wPLSR-3	Relative VarImportance
Ecological predictors					
Chlorophyll a concentration	**1.48**	**-0.34**	0.27	0.25	0.40
Mean EVI	**1.35**	**0.32**	-0.09	**0.34**	**1**
Relative density	**1.27**	**-0.32**	-0.30	0.12	**1**
Conductivity	**1.27**	-0.29	0.15	**0.34**	
Temperature seasonality	**1.07**	-0.06	0.26	-0.26	0.68
Precipitation seasonality	0.95	-0.23	0.11	0.10	
EVI seasonality	0.91	-0.23	-0.11	-0.18	
Annual precipitation	0.88	0.21	-0.12	0.30	
Annual mean temperature	0.86	-0.30	-0.03	0.13	
Water temperature	0.82	**-0.35**	-0.02	0.05	
Capture date	0.79	-0.11	-0.18	0.18	
Competitor richness	0.73	-0.08	0.27	-0.18	
Predator richness	0.72	-0.02	0.24	-0.17	
Altitude	0.68	0.04	0.26	-0.24	
Potential Evapotranspiration	0.53	-0.28	0.10	-0.02	
NO_3 concentration	0.34	-0.10	0.01	0.14	
pH	0.14	-0.06	0.03	-0.02	
(Phylo)genetic predictors					
nad4-PV6	**1.73**	-0.01	**0.39**	**0.36**	0.09
F_{ST}-PV22	**1.18**	-0.27	-0.20	-0.14	0.53
F_{ST}-PV4	0.92	0.10	0.13	**0.33**	
nad4-PV14	0.76	0.16	0.17	0.16	
F_{ST}-PV2	0.60	-0.06	**-0.47**	0.05	
R^2 by the PLSR component		0.626	0.212	0.057	

Table 2 Predictor weights for selected components of partial least squares regression (wPLSR) of male mean snout-to-vent length (SVL) of Iberian newts and their variable importance for projection (VIP), and relative variable importance (Relative VarImportance) in Akaike information criterion models. R^2 is the proportion of the original variance of male mean SVL explained by each PLSR component. Bold lettering denotes predictors with explained information (square of predictor weights*100) > 10% or VIP > 1.

Predictor variable	VIP	wPLSR-1	Relative VarImportance
Ecological predictors			
Potential Evapotranspiration	**1.72**	**-0.41**	0.28
Annual mean temperature	**1.62**	**-0.38**	0.60
EVI seasonality	**1.41**	**-0.33**	0.43
Chlorophyll a concentration	**1.18**	-0.28	
Mean EVI	**1.04**	0.24	0.08
Precipitation seasonality	**1.03**	-0.24	0.08
Annual precipitation	**1.02**	0.24	0.40
Capture date	0.65	0.15	
Temperature seasonality	0.65	-0.15	
pH	0.62	0.15	
NO_3 concentration	0.59	-0.14	
Conductivity	0.57	-0.13	
Water temperature	0.54	-0.13	
Competitor richness	0.39	-0.09	
Relative density	0.18	-0.04	
Predator richness	0.10	0.02	
Altitude	0.02	0.00	
(Phylo)genetic predictors			
F_{ST}-PV11	**1.87**	**-0.44**	0.81
R^2 by the PLSR component		0.498	

DISCUSSION

The complex interdependence among ecological factors and other selective forces along the evolutionary history of species suggests that ecogeographical rules result from different mechanisms acting individually or synergistically (Mayr, 1956; Lomolino et al., 2006). Therefore, the study of patterns and processes of body size variation has led to a prolific but chaotic research field (Huston & Wolverton, 2011). Here we considered local and macroscale predictors linked to alternative non-exclusive hypotheses explaining geographical body size variation. We found similarities and differences in the response of female compared to male Iberian newts to environmental and climatic predictors, after accounting for genetic structure. The differences existed in the magnitude rather than the direction of the relationships. For females, our results suggest that conspecific density, competition interference and resource availability influence body size variation, supporting the hypothesis of density-dependent resource availability. For males, primary production seasonality was one of the most relevant predictors of body size variation. Additionally, the direction of predictors with moderate contribution points to alternative non-exclusive hypotheses, such as heat conservation, temperature-size rule or seasonality. Overall, by combining multiple hypotheses-related predictors, using PLSR and information-theoretic approaches, our study provides new insights into the complexity of geographical body size variation.

The effect of ecological, climatic and social factors may be sex dependent (Shine, 1989). Our findings show striking differences between the sexes with regard to the implications of conspecific density and food availability. Female body size was negatively and positively associated with relative density and mean primary productivity (a proxy of food availability) respectively, while only the seasonality of primary productivity exerted a negative effect on male body size. In amphibians, it is well established that density-dependent mechanisms affect growth in larval and/or adult phases (e.g., Petranka & Sih, 1986; Denton & Beebee, 1993). Recent studies suggest that body size variation may result from reduced foraging efficiency under higher intra and/or inter-specific competition interference for acquiring food resources (Green & Middleton, 2013; Ousterhout et al., 2015). Green and Middleton (2013) showed that density-dependent competition interference in adult Fowler's toads led to smaller body size at the intra-specific level over a temporal scale of 23 years in one population. (Ousterhout et al., 2015) found that density-dependent competition interference, at least at the inter-specific level, in three *Ambystoma* salamander species might reduce foraging efficiency and lead to small larval body sizes over a portion of the species ranges. Here we provide novel evidence of a sex-specific density-dependent competition effect on adult body size variation at the intra-specific level over a species' entire geographic range. Our findings at a geographical scale (encompassing the species' range) are in line with the hypothesis of density-dependent resource availability proposed by Green and Middleton (2013) at a temporal dimension for a single population.

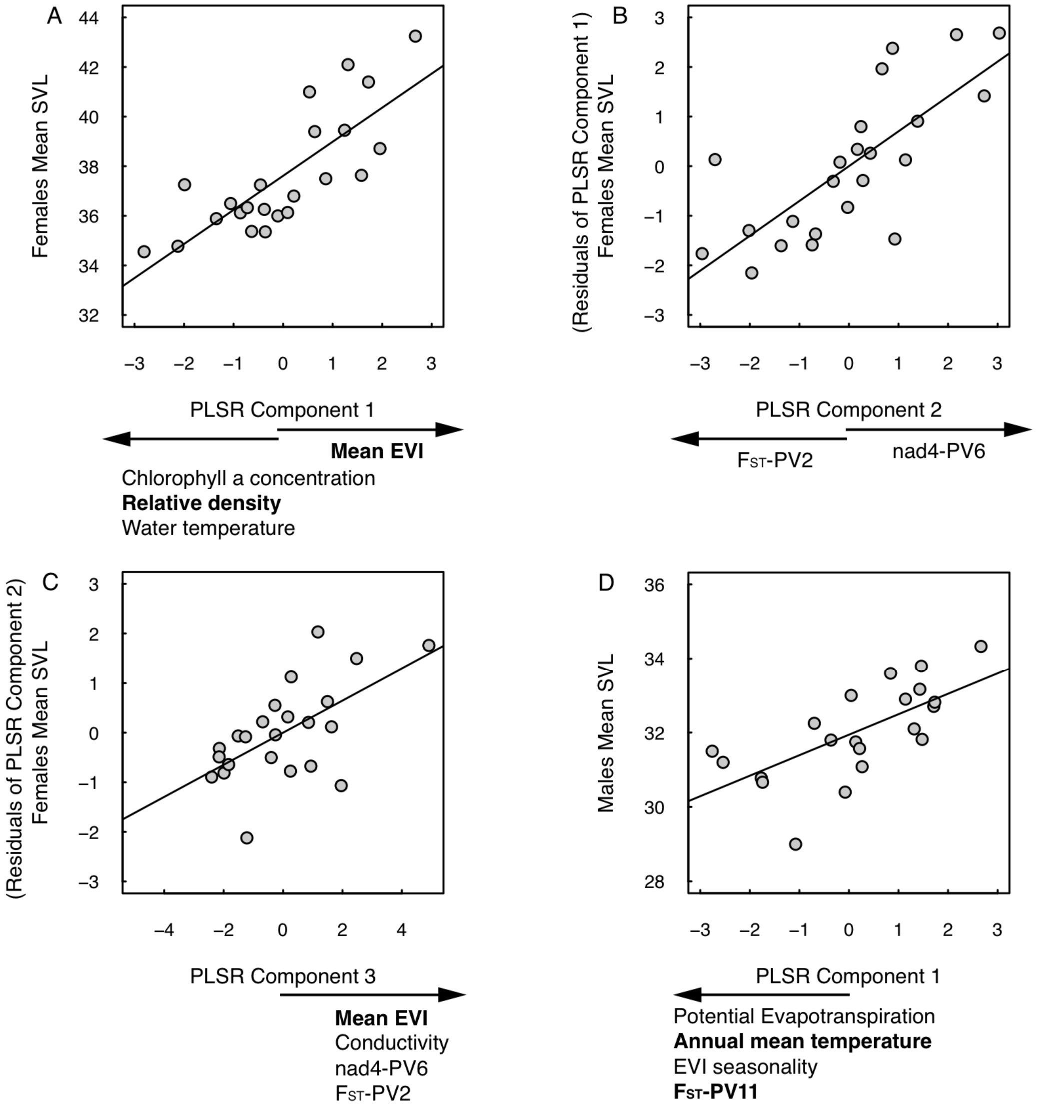

Figure 9 Relationships between mean female snout-to-vent length (SVL) of Iberian newts and the first (**a**), second (**b**) and third (**c**) relevant partial least squares regression (PLSR) component; and the relationship between mean male SVL of Iberian newts and the first (**d**) relevant PLSR component. Predictors with explained information > 10% in each PLSR component and variable importance for projection (VIP) > 1 are shown in the x-axes. Bold lettering denotes predictors with higher relative variable importance in Akaike information criterion models (see Tables 1 and 2). Sample sizes are 23 and 22 populations for females and males, respectively, across the distributional range of *Lissotriton boscai*.

Differences between females and males may result from sex-specific foraging strategies and efficiency. Specifically, sexes in this species use information differently to obtain food. Females of *L. boscai* use the foraging behaviour of conspecific females as a cue to detect food, leading to increased competition among females foraging together. However, males use trial-error tactics to detect food (regardless of the feeding activities of conspecific males), leading to a lower degree of direct intra-specific competition

(Aragón, 2009b). In agreement with this, another experiment with this species has shown that changes in the social environment uncoupled behavioural syndromes in females but not in males (Aragón, 2011). Moreover, when same-sex individuals were experimentally paired, females exhibited stronger agonistic interactions than males during feeding activities (Aragón, 2009b), whereas males only showed interference behaviour during courtship (Faria, 1995; Aragón, 2009a). Finally, seasonality of productivity at the macroscale was of greatest relevance in explaining male size. This is consistent with adult males arriving at the aquatic habitat earlier than females (Faria, 1995; Caetano & LeClair, 1999), and hence being subjected to differences in the temporal availability of food. Precipitation seasonality (i.e., ephemeral ponds) and temperature seasonality (i.e., fluctuating thermal suitability) had similar although lesser effects.

Although chlorophyll concentration, temperature, and seasonality of temperature and precipitation differed between sexes in the magnitude of their relationship, all were associated with a small body size in both sexes (Tables 1 and 2). The negative relationship between primary productivity at the local scale (i.e., chlorophyll a concentration) and body size in females and males might be explained by local eutrophication, causing reduced growth rates (Camargo & Alonso, 2006). Interestingly, the effect of chlorophyll concentration differed in direction from PLSR-1 to PLSR-2 components in females. Since the PLSR-2 component works on the residual variation not explained by the first one, a positive effect of chlorophyll on body size (as expected from the primary productivity hypothesis) may arise after removing the negative effect of chlorophyll in the PLSR-1 component. We also found a negative relationship between temperature and body size, in line with the heat conservation hypothesis and with observations in other urodele species, where individuals from populations in colder areas exhibit larger body sizes (Ashton, 2002; Ficetola et al., 2010). However, the compliance of this pattern with the heat conservation mechanism in urodeles remains questionable. For instance, according to the heat-balance hypothesis proposed by Olalla-Tárraga and Rodríguez (2007), an active thermoregulation explanation would not hold for urodeles (mostly thermoconformers). This negative relationship is also consistent with the temperature-size rule. A previous study showed that despite individuals of *L. boscai* from northern populations being older and larger, body size was not correlated with age in any population (Díaz-Paniagua & Mateo, 1999). Moreover, these authors found that smaller sizes can be observed in older individuals, which might be due to the influences of many other overriding factors. Therefore, although demographic structure may play a role in shaping life-history traits in amphibian populations, it is unlikely that only longevity explains differences in body size, and, indeed, previous evidence shows that ecological and climatic predictors are of greater relevance (Ficetola et al., 2010; Green & Middleton, 2013).

Few geographical body size variation studies have addressed local and macroscale predictors simultaneously (Meiri et al., 2007; Romano & Ficetola, 2010). Local predictors can provide information about key physiological, behavioural and ecological traits. Meiri et al. (2007) suggested that improving autoecological knowledge of species is crucial to obtain better understanding of patterns and processes underlying body size variation. Moreover, these authors highlighted that resource availability predictors might be better descriptors than other commonly used ecological and climatic predictors. While food availability and presence of competitors have been considered chief predictors in body size clines (McNab, 1971; Olson et al., 2009), population density has rarely been considered in geographical body size variation studies. The influences of food availability, density and competition might be more important for amphibian intra and inter-specific body size variation than other ecological or climatic parameters. This could explain the contradictory body size relationships in this group (Ashton, 2002; Adams & Church, 2008) and why climatic parameters poorly predict body size in amphibians (e.g., Green & Middleton, 2013).

Body size variation in response to changing ecological or climatic conditions can be mediated through genetic and/or non-genetic factors (Aragón & Fitze, 2014; Caruso, Sears, Adams & Lips, 2014; Ficetola et al., 2016). Specifically, density-dependent body size changes related to resource availability in the Iberian newt may be (partially) explained by phenotypic plasticity. Therefore, we considered inter-population genetic structure by using two different and complementary molecular markers, which provide information at two evolutionary temporal scales. Mitochondrial DNA sequences are useful to infer past evolutionary events in *L. boscai*, reflected in geographically structured phylogenetic inertia arising from old, multiple refugia (Martínez-Solano et al., 2006). Microsatellite loci are more useful to identify recent gene flow, as a result of their highly polymorphic nature and fast mutation rate (Freeland et al., 2011 and references therein). Similarly, phenotypic plasticity may be responsible for rapid temporal body size variation in response to density and food availability in adult Fowler's toads (Green & Middleton, 2013). However, we cannot discard other processes, such as phylogenetic inertia or local adaptation. At least one mitochondrial phylogenetic factor was relevant in our PLSR, suggesting a partial role of phylogenetic inertia in body size variation in *L. boscai*. Interestingly, this mitochondrial phylogenetic inertia was expressed by a PLRS component different from the PLSR component associated to relevant ecological factors. Given that PLSR components are orthogonal, the phylogenetic inertia acting here is likely independent of contemporary ecological factors. This agrees with a previous study in a vertebrate ectotherm from the same glacial refugia, where variation partitioning revealed that mitochondrial phylogenetic inertia affected body size independently of ecological factors (Aragón & Fitze, 2014). However, the relevance of two genetic factors from microsatellites in PLSR is compatible with shorter-term evolutionary processes, and it might indirectly reflect local adaptation (Kawecki & Ebert, 2004) or a genetic-based adaptive phenotypic plasticity varying at the inter-population level (Agrawal, 2001). For instance, important larval life-history traits show adaptive genetic differentiation between amphibian populations along altitudinal or latitudinal gradients, allowing faster larval development and growth to face seasonal constraints but often resulting in a smaller size at metamorphosis and adulthood (Laugen, Laurila, Räsänen & Merilä, 2003; Luquet, Lena, Miaud & Plenet, 2015).

To conclude, key variables associated with geographic body size variation in an urodele amphibian are conspecific density and primary productivity, supporting the density-resource availability hypothesis. These relationships were sex-specific and in line with experimental evidence of sex-dependent use of social information on conspecific feeding activities. Future experiments should consider explicitly the interaction of inter-population variation of competition with other relevant environmental parameters. More generally, we advocate for geographic phenotypic variation studies encompassing different (phylo)genetic and ecological spatio-temporal scales, with analytical designs addressing the interdependence among predictors. Finally, correlative studies will benefit from selecting model species with a well-established experimental background to make better inferences about evolutionary and ecological processes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the APPENDICES

Appendix 1.1 Spatial autocorrelation in models

Appendix 1.2 mtDNA marker sequencing and phylogenetic analyses

Appendix 1.3 Microsatellites, genetic diversity and population differentiation

BIOSKETCH

Miguel Peñalver-Alcázar is interested in the study of patterns and processes along geographical and environmental gradients using amphibians and reptiles as model species.

Author contributions: P.A and M.P.A designed the study; M.P.A performed the field sampling, collected the data and performed all the analyses; F.S. performed mtDNA sequencing and microsatellite genotyping; I.M.S and F.S. guided and contributed to discuss phylogeographic and microsatellite analyses. M.P.A and P.A led the writing and I.M.S and F.S reviewed and commented the paper.

CHAPTER II

Assessing Rensch's rule in a newt: roles of primary productivity and conspecific density in interpopulation variation of sexual size dimorphism

Miguel Peñalver-Alcázar, Pedro Galán & Pedro Aragón

Under review

ABSTRACT

Aim

Major efforts have been devoted to understand the geographic pattern of sexual size dimorphism (SSD). Rensch's rule poses that SSD increases with body size in male-biased SSD species and decreases with body size in female-biased SSD species. This pattern and its inverse have been mainly explored at the interspecific level, whereas research at the intraspecific level has been largely neglected until recently. Here we test whether the allometric pattern of SSD in an urodele amphibian conforms to Rensch's rule and evaluate the relative role of four potential mechanisms: sexual selection, fecundity selection, density-dependent resource availability and differential plasticity.

Location

Iberian Peninsula.

Taxon

Lissotriton boscai (Amphibia: Caudata: Salamandridae).

Methods

We used original (field-based) and published data on body size, courtship behaviour and fecundity parameters and a suite of climatic, ecological and genetic (mitochondrial and nuclear markers) predictors to assess these hypotheses.

Results

The results showed that interpopulation variation of SSD increased with increasing female mean body size, supporting the inverse Rensch's rule pattern. Primary productivity-related variables and female density were among the most relevant ecological predictors of SSD after accounting for genetic structure and temporal autocorrelation.

Main conclusions

This study reveals that an interface between the density-dependent resource availability hypothesis and the differential plasticity hypothesis explains the inverse Rensch's rule. We discuss how combining biogeographical and experimental approaches can provide alternative interpretations to the classical sexual and fecundity selection hypotheses on the interpopulation variation in SSD.

Keywords: density-dependent resource availability, differential plasticity hypothesis, intraspecific competition, intraspecific level, primary productivity, Rensch's rule, Sexual size dimorphism, urodele amphibian

INTRODUCTION

Biologists have long been interested in documenting patterns of sexual size dimorphism (SSD) and understanding their underlying ecological and evolutionary processes. SSD is common throughout the animal kingdom and is defined as the body size difference between adult females and males of a species (or population), where one sex is larger than the other (Andersson, 1994; Fairbairn, 1997). Rensch's rule describes an allometric relationship between SSD and body size among species, considering that body size divergence is greater among males than in females. Therefore, the degree of SSD increases with body size in species with male-biased SSD and decreases with body size in species with female-biased SSD (Rensch, 1950; Abouheif & Fairbairn, 1997; Fairbairn, 1997). Several studies provide evidence in species showing a male-biased SSD (e.g., Fairbairn, 1997; Cox, Skelly & John-Alder, 2003; Székely, Freckleton & Reynolds, 2004). However, evidence remains scarce in female-biased SSD species (Webb & Freckleton, 2007; Liao, Zeng, Zhou & Jehle, 2013), and exceptions exist showing the inverse of Rensch's rule, i.e., the degree of SSD increases with female body size, among female-biased SSD ectotherms (Herczeg, Gonda & Merilä, 2010; Liao, Liu & Merilä, 2015).

Four leading hypotheses explain the occurrence of SSD and the existence of Rensch's rule or it's inverse: (1) The sexual selection hypothesis poses that sexual selection acts more strongly in one sex, followed by a simultaneous but weaker correlational selection in the other sex (Abouheif & Fairbairn, 1997; Fairbairn, 1997). Therefore, strong sexual selection acting on males (towards small or large body size) is expected to lead to a pattern of increasing SSD with body size in male-biased SSD species consistent with Rensch's rule, whereas strong sexual selection acting on females should lead to the inverse Rensch's rule, increasing SSD with body size in female-biased SSD species; (2) The fecundity selection favouring predicts that fecundity selection favours larger females due to their higher reproductive output (e.g., larger clutches or eggs), leading to increasing SSD with body size in female-SSD biased species and decreasing SSD with body size in male-SSD biased species, i.e., a pattern consistent with the inverse Rensch's rule (e.g., Fairbairn, 1997; Herczeg et al., 2010; Liao, 2013); (3) The differential plasticity hypothesis poses that sex-specific plastic responses to environmental factors lead to interpopulation variation in the degree of SSD (Fairbairn, 2005). Specifically, SSD would evolve following a pattern consistent with Rensch's rule when males show greater phenotypic plasticity than females, whereas patterns conforming to the inverse Rensch's rule would emerge under female-biased plasticity; (4) The density-dependent resource availability hypothesis suggests SSD may result from male and female body size being differently affected by resource availability, modulated by conspecific density (Green & Middleton, 2013; Peñalver-Alcázar, Martínez-Solano, Sequeira & Aragón, 2017), e.g., due to sex-specific resource requirements for mating and reproduction (Colwell, 2000). Thus, resource limitation may be a key driver of SSD patterns under high population density (Garel, Solberg, SÆther, Herfindal & Høgda, 2006). This scenario can be seen as a case of the general differential plasticity hypothesis and would lead to the same patterns.

Bernhard Rensch framed his rule at the subspecies, genus and/or family levels (Abouheif & Fairbairn, 1997). Accordingly, research was conducted mainly at the interspecific level (Fairbairn, 1997; Colwell, 2000; Cox et al., 2003; Székely et al., 2004; Colleoni, Denoël, Padoa-Schioppa, Scali & Ficetola, 2014). While the study of Rensch's rule recently moved towards the intraspecific level, these studies are still scarce (Fairbairn, 2005; Blanckenhorn, Stillwell, Young, Fox & Ashton, 2006; Lengkeek et al., 2008). In amphibians, where females are generally larger than males (Shine, 1979), interspecific studies provided contrasting results (De Lisle & Rowe, 2013; Liao et al., 2013; Nali, Zamudio, Haddad & Prado, 2014). At the intraspecific level, the inverse Rensch's rule has been described in several female-biased SSD anuran and urodele species (Ivanović, Sotiropoulos, Furtula, Džukić & Kalezić, 2008; Liao, 2013; Liao et al., 2015), although exceptions exist (Sinsch, Pelster & Ludwig, 2015). Interpopulation variation in SSD has been related to a strong fecundity selection for larger females in anurans (Liao, 2013; Liao et al., 2015) and suggested for newts (Malmgren & Thollesson, 1999; Ficetola et al., 2010). Overall, results appear to depend on the phylogenetic level and species group, leading to an unclear pattern of variation of SSD in amphibians and disagreement about the underlying processes.

This study investigates patterns and processes of interpopulation variation in SSD by focusing on an intraspecific test of Rensch's rule in the Iberian newt (*Lissotriton boscai*). We used literature and field data on populational body size for female and male newts to first explore whether the relationship between body size and SSD follows Rensch's rule or its inverse. Then, we tested the relationship of a sexual dimorphism index (SDI) with different local and macroscale environmental predictors and (phylo)genetic factors derived from field populations to assess the relative role of four leading mechanisms related to interpopulation variation in SSD, namely: sexual selection, fecundity selection, density-dependent resource availability and differential plasticity. We also performed complementary analyses retrieving experimental data from previous studies to further elucidate the role of these hypotheses. A recent study on *L. boscai* showed that density-dependent body size changes related to resource availability can be partially explained by sex-specific phenotypic plasticity (Peñalver-Alcázar et al., 2017). Therefore, we predict that the differential plasticity hypothesis for the SSD (Fairbairn, 2005) may hold, at least partially, after controlling for genetic factors.

MATERIAL AND METHODS

Study species

Lissotriton boscai (Lataste 1879) is a small-bodied urodele endemic to the western part of the Iberian Peninsula, found from sea level up to 1870 meters (Fig. 10). This is one of the most aquatic newts and preferred aquatic habitats include shallow streams and rivers, natural and artificial ponds, watering troughs and water springs (Lizana, Perez-Mellado & Ciudad, 1990). Densities are higher in northern and central populations than in southern and easternmost ones (García-París, Montori & Herrero, 2004). Populations are divided into two geographically–structured genetic lineages, each containing three sub-clades (Martínez-Solano, Teixeira, Buckley & García-París, 2006; Teixeira et al., 2015). Female oviposition may last from three or four months up to the entire aquatic phase, and egg size is not related to female body size (Díaz-Paniagua, 1986; Brea, Galán, Ferreiro & Serantes, 2007). Experimental and observational evidence revealed that conspecific attraction (Aragón, López & Martín, 2000) and competitive interactions occur mainly amongst females during feeding activities and amongst males during courtship (Faria, 1995; Aragón, 2009a, b).

Data collection

We collected data on body size (length from the snout to the posterior margin of the cloaca slit, snout-vent length, SVL) both through fieldwork and a literature review, representing 2108 individuals from 42 populations of *L. boscai*. Field studies were performed in the springs of 2013 and 2015 during which 27 populations were sampled. Seventeen of those populations were sampled over two years, 5 only in 2013, and 5 only in 2015 (Fig. 10 and Appendix 2.1, Table A2.1). Adult newts were captured using a dip net, sexed and measured for SVL to the nearest millimetre. In 2013, a small portion of the tail tip was also collected for genetic analysis (see Peñalver-Alcázar et al., 2017). Sampled populations entailed small traditional watering troughs for cattle, small watering spring for humans, small natural and human-made ponds as well as small pools of shallow streams and rivers with slow current. The populations' reduced dimensions allowed us to sample each aquatic habitat several times, capturing as many newts as possible. In the few populations with vegetated margins, (3 natural and artificial ponds), we also screened these areas for newts. Sampling was always performed between mid-late morning and late evening, since aquatic adult newts are mainly active during daylight and twilight (García-París et al., 2004). The sampling effort for each population was recorded to calculate capture rate as a proxy of relative female and male density following the expression number of individuals per sex / sampling effort invested at each population. Altitude and geographic coordinates were also recorded at each population. Furthermore, climatic variables (temperature and precipitation) at 1-km resolution were derived from the WorldClim database (Hijmans, Cameron, Parra, Jones & Jarvis, 2005) for the sampled populations. Only those climatic predictors related to our main hypotheses of sexual size dimorphism were selected. Monthly maps of Enhanced Vegetation Index (EVI) generated from MODIS satellite images at 1-km resolution were used as a proxy of aboveground net primary productivity (Running et al., 2004) to calculate primary productivity and primary productivity seasonality at the sampling sites for the period 2012-2013 and 2014-2015 (Peñalver-Alcázar et al., 2017). Finally, one integer variable accounting for the timing of the sampling relative to the seasonal change was calculated using the capture date of each population. This variable is expressed as the cumulative number of days within the year. It allows us to control for the potential impact of temporal autocorrelation in the statistical analyses. Overall, field samplings provided data on SVL for 1040 individuals (524 females and 516 males). Then, a literature review of the previous research on the Iberian newt provided mean SVL data for 15 additional populations representing 1068 individuals (496 females and 572 males; Appendix 2.1, Table A2.2). The literature SVL data included measures from both fresh and preserved specimens. Preserved specimens often exhibit tissue shrinkage (Verrell, 1985). Therefore, an index to correct the SVL from preserved specimens was applied following the values of tissue shrinkage described in Verrell (1985). The correction was done according to the following sex-specific formulas:

$$\text{Males - } SVL_{\text{corrected}} = SVL_{\text{preserved}} + SVL_{\text{preserved}} \text{ x } 0.015$$

$$\text{Females - } SVL_{\text{corrected}} = SVL_{\text{preserved}} + SVL_{\text{preserved}} \text{ x } 0.029$$

Most of the studies from the literature review provide neither geographical coordinates nor sampling effort. Therefore, literature-based body size data were only used for the analyses on the magnitude and direction of SSD to assess Rensch's Rule and not for the analyses about the potential underlying processes.

Sexual size dimorphism was quantified for each field population by year following the method proposed by Lovich and Gibbons (1992). Accordingly, sexual size dimorphism index (SDI) was calculated as (female mean length/male mean length) – 1. Positive index values indicate female-biased dimorphism, zero values indicate no sex differences in mean body size, and negative values indicate male-biased dimorphism. This SDI has some advantages including values that are symmetric around zero, an easy and intuitive interpretation, good statistical performance and it is commonly used in studies of SSD (Lovich & Gibbons, 1992; Smith, 1999).

Finally, (phylo)genetic factors (i.e., (phylo)genetic eigenvectors; PVs) were calculated to select and include new predictors accounting for (phylo)genetic variation and inertia in the analyses (Diniz-Filho, de Sant'Ana & Bini, 1998; Peñalver-Alcázar et al., 2017). PVs were derived first through a principal coordinate analyses based on a double-centred pairwise genetic distances matrix at the population level for two genetic markers (the mitochondrial *nad4* gene and seven polymorphic microsatellite loci) for the 22 populations sampled in 2013. Then, correlations between derived mitochondrial and microsatellite PVs and residuals of partial least squares regression (PLRS) including only ecological parameters were tested. Those PVs significantly correlated with the residuals were selected and included into further PLSR analysis (see Peñalver-Alcázar et al., 2017 for methodological details). These PVs may inform PLSR models about potential influences on SDI of genetic relationships and exchanges among populations at different spatial and temporal scales. Overall, principal coordinate analyses provided 16 mitochondrial PVs (*nad4*-PVs) and 21 microsatellite PVs (F_{ST}-PVs) of which only *nad4*-PVs 2, F_{ST}-PVs 3 and F_{ST}-PVs 11 were significantly correlated with residuals from PLSR ecological model (Pearson's $r > |0.43|$, $P < 0.05$ in all cases).

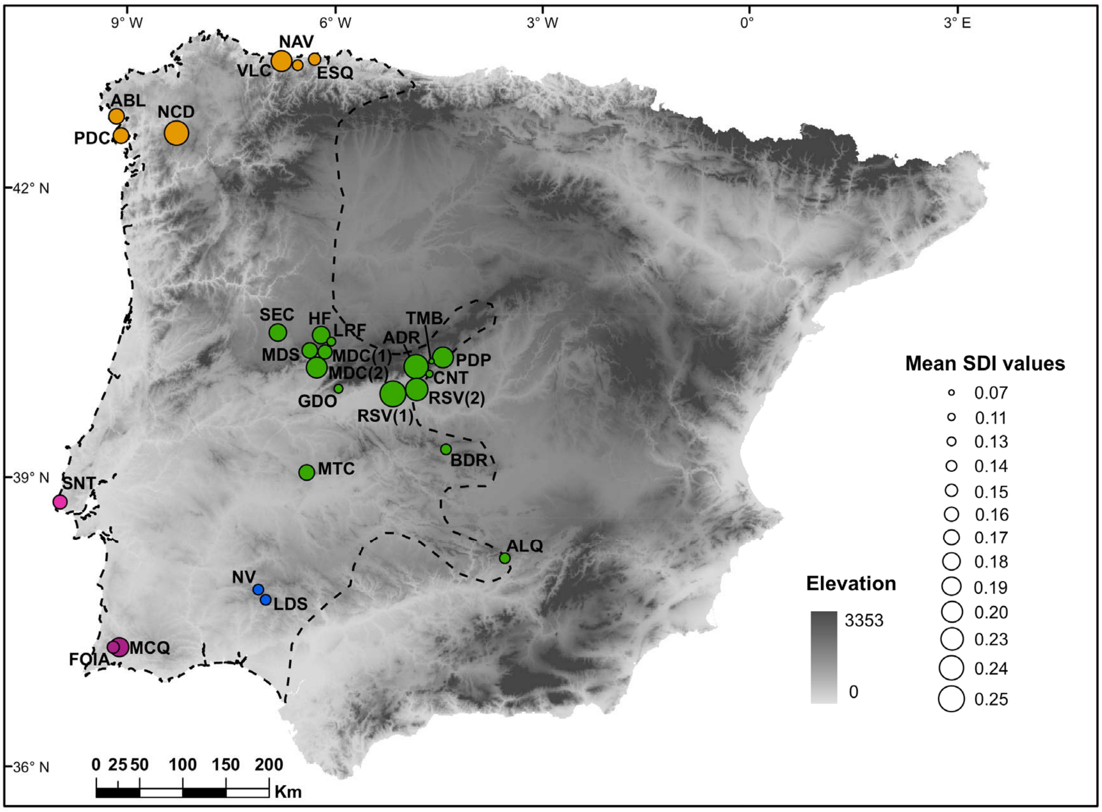

Figure 10 Distribution of the 27 populations (coloured circles) sampled during the springs of 2013 and 2015 across the range of *Lissotriton boscai* (dashed line) in the Iberian Peninsula. The background map shows elevation. Circle widths are proportional to mean sexual dimorphism index (SDI) values and colours represent the different intraspecific clades (Martínez-Solano et al., 2006; Peñalver-Alcázar et al., 2017). Population names correspond to population codes in Appendix 2.1, Table A2.1.

Statistical analyses

To test Rensch's Rule, the body size of one sex was regressed against the body size of the other sex using the population means of SVL data from each of the 42 field (2013 + 2015) and literature populations. Moreover, Rensch' rule was explored separately for the 22 populations sampled in 2013 because this dataset provided useful and detailed environmental and genetic information for further analyses. The use of Model I regression (ordinary least square regression) may be statistically incorrect because female and male body size variables are measured with error in the same scale, neither of them are fixed and both are exchangeable between the *x* and the *y* axis. Consequently, the use of Model II regression (major axis regression) may be more appropriate (Fairbairn, 1997). However, OLS may be a reliable approach to explore allometry and Rensch's rule (Kilmer & Rodríguez, 2017) and studies assessing Rensch's Rule usually report results from both approaches for comparative reasons (e.g., Herczeg et al., 2010). Therefore, Model I and II regressions were performed with the R packages 'lmodel2' (Legendre, 2014) and 'smart' (Warton, Duursma, Falster & Taskinen, 2012) on the log transformed body size variables, placing female body size in the *x* axis (as recommended by Fairbairn, 1997) and testing the null hypothesis of regression slope = 1 for the 95% confidence interval to discern between allometry and isometry in SSD. Under this hypothesis, SSD evolves following Rensch's Rule if the slope of the regression is significantly greater than 1; SSD evolves following the inverse Rensch's Rule if the slope of the regression is significantly less than 1; and SSD evolves in isometry with body size if the regression slope is not significantly different than 1.

Once we assessed the SSD pattern in *L. boscai*, three different sets of statistical analyses were performed to discern the potential mechanisms underlying SSD. First, a PLSR analysis based only on the 2013 dataset was conducted with SDI as dependent variable and relevant ecological and (phylo)genetic variables as predictors. The absence of individual-based genetic samples for 2015 prevented us to repeat the same analysis for this year. PLSR is an improvement of multiple regression analysis and overcomes limitations of commonly used multivariate procedures. This approach is recommended for dealing with high number of potentially correlated predictor variables and when the sample size is smaller than the number of predictor variables (Carrascal, Galván & Gordo, 2009). It is based on the iteratively extraction of orthogonal independent factors

(PLSR components) derived from the linear combination of the predictors and the response variable. The PLSR components maximize the covariance between the predictors and the response variable. Most of the variance in the explanatory variables is concentrated in the first few PLSR components and less important PLSR components are discarded (e.g., via cross-validation), reducing the multidimensionality. PLSR balances information both in predictors and response variables, reducing the potential effect of higher but irrelevant predictor variations (Garthwaite, 1994; Mevik & Wehrens, 2007). The interpretation of PLSR models relies on the explanatory capacity of each component (R^2), the predictor weights and the variable importance for projection (VIP). The predictor weights describe the direction and the strength of the relationship between predictors and response variable for each PLSR component. The contribution of each predictor (i.e., the information explained) within each component is calculated by means of sum of squares of the weights, because the overall sum of squares of predictors within the component is equal to one. In PLSR analyses, predictors with a VIP value larger than 1 and explained information (i.e., square of predictor weights*100) larger than 10% within at least one component were considered predictors of greater relevance for explaining SDI (Carrascal et al., 2009; Peñalver-Alcázar et al., 2017). PLSR analysis was performed using the orthogonal scores algorithm (NIPALS) and selecting the most relevant components through a cross-validation procedure (default setting 'CV' in 'pls' R package (Mevik & Wehrens, 2007). Finally, the potential existence of spatial autocorrelation was explored calculating Moran's *I* and spatial correlograms for the residuals of the PLSR model (Appendix 2.6, Fig. A2.6).

Second, Akaike's information criterion (AIC) (Burnham & Anderson, 2002) for model selection was used on the combined (2013 + 2015) field dataset. We ran linear mixed models with SDI as dependent variable, and altitude, capture season, relative females and males density, mean EVI, EVI seasonality, annual mean temperature, annual precipitation, temperature seasonality and precipitation seasonality as fixed factors, and population and year as random factors. Linear mixed models and model selection were performed using R packages 'lme4' (Bates, Maechler, Bolker & Walker, 2014) and 'MuMIn' (Bartón, 2013), respectively. To find the most parsimonious combination of fixed effects, all possible models were fitted using maximum likelihood, keeping the same random effects structure for all models (i.e., population and year).

Then, the second-order AIC corrected for small sample size (AICc) and AIC weight (AICw) scores were calculated for all possible models. The cut-off criterion of ΔAICc < 2 was used to delineate a "top model set", and then, model-averaged parameter estimates, standard errors, confidence intervals and relative variable importance (as the sum of the AICw of all selected models, Burnham & Anderson, 2004) were computed for the fixed factors in all selected models. Estimates and standard errors of weighed parameters were calculated following the "zero average method" (Burnham & Anderson, 2002; Grueber, Nakagawa, Laws & Jamieson, 2011). Prior to model building, predictors were standardized separately for both sampling years to avoid scale and unit differences, providing thus an appropriate interpretation of their relative contributions (Quinn & Keough, 2002).

Several field-sampled populations had low sample size (number of females or males per population was 4 or even 2), which might hinder drawing robust inferences from our statistical analyses. Therefore, we re-run all statistical analyses including only those populations with at least 5 individuals of each sex, i.e., the smallest included population had 5 males and 5 females (see Appendix 2.2). We additionally performed two different sets of analysis using the averages of SVL and SDI across years and sexes for populations sampled in both years (i.e., 2013 and 2015) with this minimum sample size, as often done in studies at the interspecific level (Appendix 2.3). Lastly, we reanalysed experimental data of Iberian newt courtship behaviour from Aragón (2009b) and of female fecundity parameters and SVL from Brea et al. (2007) to explore the sexual selection hypothesis and the fecundity selection hypothesis, respectively (Appendices 2.4 and 2.5). All statistical analyses were performed in R 3.0.2 (R Core Team, 2013), and spatial autocorrelation analyses in SAM 4.0 (Rangel, Diniz-Filho & Bini, 2010).

RESULTS

Model I regression showed a significant relationship between the mean sizes of the sexes across populations, and this relationship differed significantly from 1 for populations sampled in 2013 ($R^2 = 0.57$, slope = 0.50 [95% CI = 0.30-0.70], $P < 0.001$, Fig. 11a) and in the combined field and literature populations ($R^2 = 0.81$, slope = 0.78 [95% CI = 0.66-0.90], $P < 0.001$, Fig. 11b). Model II regression revealed similar results for populations sampled in 2013 ($R^2 = 0.57$, slope = 0.59 [95% CI = 0.37-0.86], $P < 0.001$, Fig. 11a) and combined field and literature populations ($R^2 = 0.81$, slope = 0.85 [95% CI = 0.73-0.99], P = 0.04, Fig. 11b).

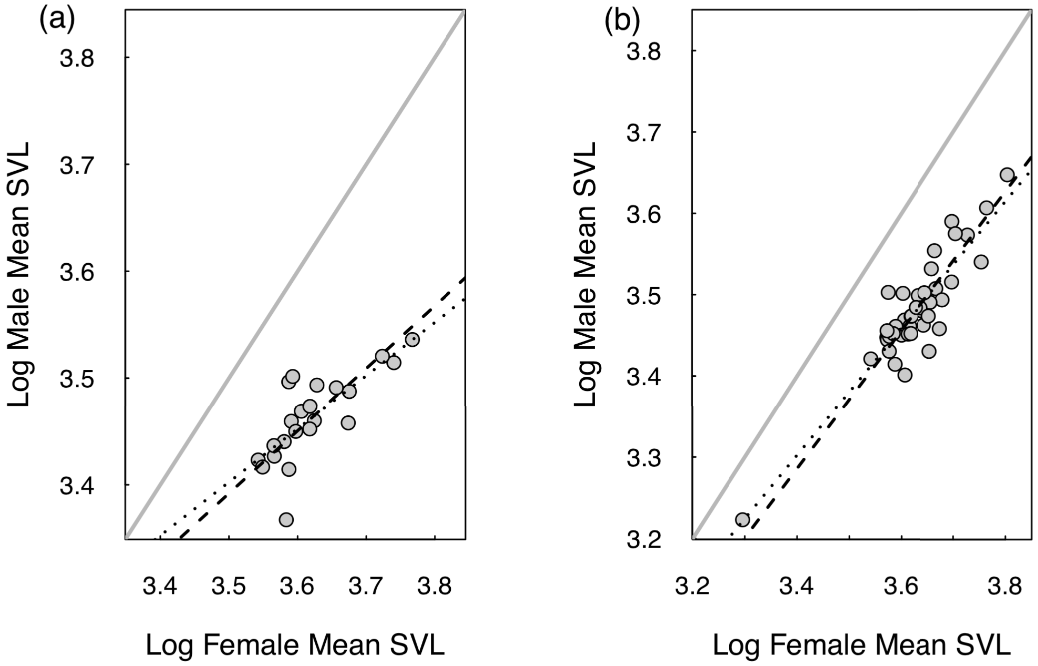

Figure 11 Log male mean snout-vent length (SVL) versus log female mean SVL for (**a**) 22 field populations of *Lissotriton boscai* sampled in 2013 with Model I regression dotted line (slope = 0.57 [95% CI = 0.30-0.70]) and Model II regression dashed line (slope = 0.59 [95% CI = 0.37-0.86]) and (**b**) 42 field (2013 and 2015) and literature populations of *L. boscai* with Model I regression dotted line (slope = 0.78 [95% CI = 0.66-0.90]) and Model II regression dashed line (slope = 0.85 [95% CI = 0.73-0.99]). The thick grey line represents isometry, i.e., slope = 1. Each dot represents a single population.

The PLSR model provided three significant components explaining 77.5% of the original variance in SDI for the 22 populations sampled in 2013 (PLRS-1: $r = 0.73$,$P < 0.0001$; PLRS-2: $r = 0.50$, $P = 0.018$; PLRS-3: $r = 0.59$, $P = 0.004$; Table 3). Mean EVI, relative female density, EVI seasonality and capture season were the most relevant ecological predictors for explaining SDI according to VIP (> 1 in 4 out of 10 cases, Table 3) and information explained in the PLSR-1 (> 10% in 3 out of 10 cases, Table 3), PLSR-2 (> 10% in 2 out of 10 cases, Table 3) and PLSR-3 (> 10% in 1 out of 10 cases, Table 3). Annual precipitation, temperature seasonality and altitude showed a moderate influence, since their VIP was < 1 and explained information was only > 10% in PLSR-3 (Table 3). The rest of the ecological predictors were not considered relevant according to VIP (< 1 in all cases) and information explained in PLSR (< 10% in all cases). Furthermore, (phylo)genetic predictors were also relevant to explain SDI, since *nad4*-PV2, F_{ST}-PV3 and F_{ST}-PV11 had a VIP > 1 or explained information > 10% in the PLSR components (Table 3). Finally, residuals of the PLSR model did not show spatial structure based on Moran's *I* correlograms (Appendix 2.6, Fig. A2.6).

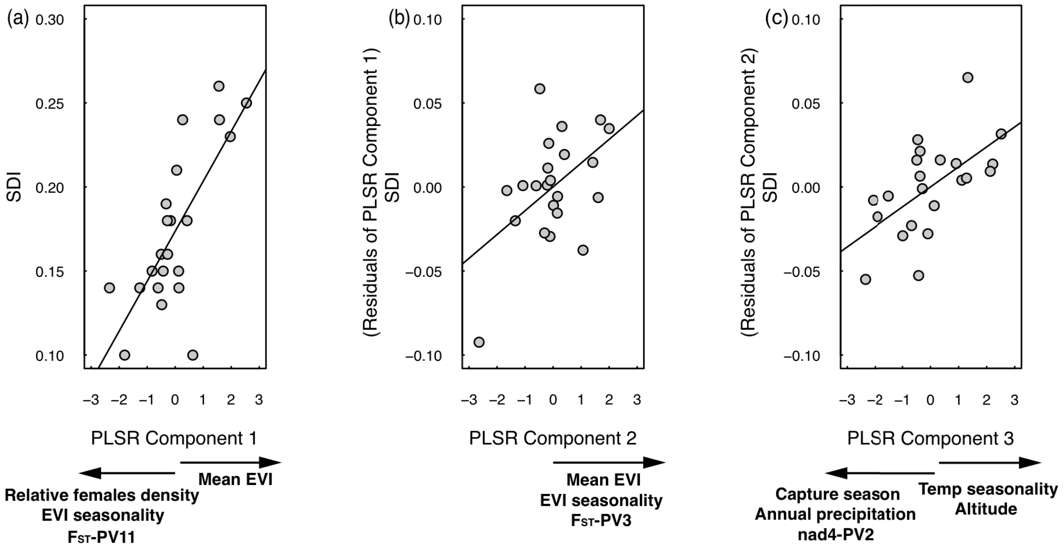

Figure 12 Relationships between sexual dimorphism index (SDI) for 22 field populations of the Iberian newt sampled in 2013 and the first (**a**), second (**b**) and third (**c**) relevant partial least squares regression (PLSR) components. Predictors with explained information > 10% in each PLSR component and variable importance for projection (VIP) > 1 are shown in the x-axes (see Table 3). Each dot represents a single population.

Mean EVI showed a positive relationship with the response variable in PLSR-1 and PLSR-2, whereas EVI seasonality was negatively and positively related with the response variable in PLSR-1 and PLSR-2, respectively (Fig. 12a,b). Both primary productivity-related variables were amongst the most relevant predictors for SDI (Table 3). However, relevance of mean EVI was greater (VIP = 1.6, explained information in PLSR-1 = 18.9% and in PLSR-2 = 35.9%, Table 3) than EVI seasonality (VIP = 1.3, explained information in PLSR-1 and PLSR-2 = 14.5% in both cases, Table 3). The contribution of capture season was noticeable but was consigned to the last PLSR component (VIP = 1.1, explained information in PLSR-3 = 18.3%, Fig. 12c). Relative female density was a highly relevant predictor of SDI (VIP = 1.6, Table 3) and showed the strongest negative relationship with SDI in PLSR-1 (explained information in PLSR-1 = 26.4%, Table 3, Fig. 2a). This result contrasted with the lack of relevance of relative male density (VIP = 0.9 and information explained in PLSR < 10% in all three components, Table 3).

Model selection based on Akaike's information criterion for the combined 2013 + 2015 dataset provided 13 models for model averaging according to the cut-off criterion of ΔAICc < 2 (Appendix 2.7, Table A2.6). Mean EVI and capture season were the most relevant parameters for explaining SDI, and were present in all selected models (relative importance = 1 in both cases, Table 4). Relative female density was also relevant, being present in more than half of the models selected (relative importance = 0.620, Table 4). The rest of the predictors were present in less than half of the selected models and therefore were not considered relevant (relative importance < 0.5 in all cases). Capture season and relative female density were negatively related to SDI (estimate ± SE = -0.028 ± 0.008 and -0.008 ± 0.008, respectively), whereas mean EVI was positively related to SDI (estimate ± SE = 0.030 ± 0.008).

The results of the analyses excluding populations with low sample size as well as the analyses using averages of SVL and SDI across years and sexes for populations sampled in both years were in agreement with those derived from the analyses performed with the complete dataset (Appendix 2.2 and 2.3). The analyses of the data from Aragón (2009b) and Brea et al. (2007) revealed that male body size is not related to female interest during male courtship displays (Appendix 2.4), and that female body size is not related to parameters of fecundity (Appendix 2.5).

Table 3 Predictor weights for selected components of partial least squares regression (wPLSR) of sexual dimorphism index (SDI) for 22 field populations of the Iberian newt sampled in 2013 and their variable importance for projection (VIP). R^2 is the proportion of the original variance of SDI explained by each PLSR component. Bold lettering denotes predictors with explained information (square of predictor weights*100) > 10% or VIP > 1.

Predictor variable	VIP	wPLSR-1	wPLSR-2	wPLSR-3
Ecological predictors				
Mean EVI	**1.6**	**0.44**	**0.60**	-0.13
Relative female density	**1.6**	**-0.51**	0.12	0.06
EVI seasonality	**1.3**	**-0.38**	**0.38**	0.29
Capture season	**1.1**	-0.30	-0.23	**-0.43**
Relative male density	0.9	-0.28	0.20	-0.14
Annual precipitation	0.8	0.18	0.27	**-0.33**
Temperature seasonality	0.6	-0.01	-0.05	**0.44**
Altitude	0.6	0.02	-0.13	**0.40**
Annual mean temperature	0.4	-0.09	0.23	-0.06
Precipitation seasonality	0.4	-0.13	0.03	0.01
(Phylo)genetic predictors				
nad4-PV2	**1**	-0.27	-0.06	**-0.46**
F_{ST}-PV11	**1**	**-0.31**	0.24	0.12
F_{ST}-PV3	0.6	-0.06	**0.43**	0.03
R^2 by the PLSR component		0.540	0.114	0.121

Table 4 Summary results of the AIC model selection procedure of linear mixed models for sexual dimorphism index (SDI) in *Lissotriton boscai* for 2013 and 2015 combined data after model-averaging the most parsimonious models according to the cut-off criterion of ΔAICc < 2. Model-averaged parameters standardized estimates were calculated using the "zero-average method", with the unconditional standard errors (SE) providing model selection uncertainty and the lower and upper confidence intervals (CI). The Relative importance was calculated as the sum of the AIC weight of all selected models (a value of 1 indicates that parameter was present in all of the averaged models, N = 13).

Parameter	Estimate	SE	Lower CI	Upper CI	z-value	Relative importance	N
(Intercept)	0.166	0.006	0.154	0.178	27.489		
Capture season	-0.028	0.008	-0.044	-0.013	3.645	1.000	13
Mean EVI	0.030	0.008	0.013	0.046	3.497	1.000	13
Relative female density	-0.008	0.008	-0.024	0.008	0.983	0.620	8
EVI seasonality	0.006	0.008	-0.010	0.021	0.716	0.430	5
Annual mean temperature	-0.005	0.008	-0.021	0.011	0.597	0.360	5
Altitude	0.006	0.009	-0.013	0.025	0.612	0.360	5
Precipitation seasonality	0.002	0.005	-0.009	0.013	0.355	0.150	2
Annual precipitation	0.002	0.006	-0.011	0.015	0.325	0.150	2

DISCUSSION

We investigated the relationship between sexual size dimorphism (SSD) and body size across the geographical range of an urodele amphibian, *Lissotriton boscai*, and explored local and macroscale environmental predictors, (phylo)genetic factors and courtship behaviour responses potentially linked to four hypotheses concerning SSD evolution. We found that the degree of SSD increased with female mean body size, supporting the inverse of Rensch's rule. Furthermore, mean EVI, EVI seasonality and relative female density were the most relevant ecological predictors related to SSD after accounting for genetic relationships among populations and temporal autocorrelation. These results reveal a leading role of resource limitation coupled with population density in shaping interpopulation SSD variation in *L. boscai*, and a minor role of (phylo) genetic factors.

Support for Rensch's rule comes mainly from interspecific studies in species with male-biased SSD (Abouheif & Fairbairn, 1997; Fairbairn, 1997; Colwell, 2000; Székely et al., 2004; Dale et al., 2007), and some studies suggest compliance in female-biased SSD species (Fairbairn, 1997; Székely et al., 2004). Comparatively, research at the intraspecific level in species where females are the larger sex is scarce, and shows variation of SSD consistent with the inverse of Rensch's rule across taxa (Fairbairn, 1997; Teder & Tammaru, 2005; Herczeg et al., 2010). Likewise, our findings point to an inverse Rensch's rule, and agree with recent intraspecific studies where the degree of SSD increases with female body size in anurans and urodeles with female-biased SSD (Ivanović et al., 2008; Liao, 2013; Liao et al., 2015). However, the interpopulation variation in SSD observed in *L. boscai* contrasts with an interspecific study using 52 salamandrid species (Colleoni et al., 2014), where no allometric relationship in body size for female-biased SSD species (including *L. boscai*) was found, thus ruling out the inverse of Rensch's rule. Therefore, SSD patterns at the intraspecific level do not necessarily match those at the interspecific level, suggesting that distinct underlying processes may act.

Because environmental factors and evolutionary constraints act alone or in combination throughout a species´ evolutionary history, biological rules may not have a simple explanation (Mayr, 1956). Rensch's rule is no exception and sexual selection, fecundity selection and natural selection may account for allometric patterns of SSD (Fairbairn, 1997). Our findings reveal that ecological factors related to resource availability and conspecific density play a relevant role in shaping SSD in *L. boscai*. Sex-specific differences in body size (i.e., sexual dimorphism index, SDI) decreased as primary productivity declined and the seasonality of primary production increased. A limited resource supply has been suggested as a mechanism behind a smaller size in both sexes and a reduced SSD in flower mites and hummingbirds (Colwell, 2000), where sexual selection favouring energetically-conservative smaller males is followed by a potential stabilizing selection on females. However, Colwell (2000) found a pattern of allometry consistent with Rensch's rule, which contrasts with our findings, suggesting that the constraints imposed by low resource availability may be stronger in *L. boscai* females than in males. Similarly, Teder and Tammaru (2005) showed that females were more sensitive than males to environmental factors in insect species with female-biased SSD. Moreover, the fact that relative female density was negatively related to SDI suggests a potential interplay between resource limitation and conspecific density. This is in line with a study suggesting that resource limitation and adult sex ratio drive SSD in Norwegian moose (*Alces alces*) populations (Garel et al., 2006). In concordance, previous findings on geographical body size variation in *L. boscai* have shown that females are strongly and negatively affected by lower primary productivity and higher conspecific density, resulting from higher intraspecific competition amongst females than amongst males (Aragón, 2009a; Peñalver-Alcázar et al., 2017). Therefore, we propose that females attain larger sizes in populations under favourable environmental conditions (i.e., sufficient food availability and lower female abundance), resulting in an increased degree of SSD, whereas in poorer environmental conditions (stronger) constraints on female body size lead to a lower SSD.

Most studies assessing Rensch's rule suggest that SSD evolves through sexual selection acting on male body size coupled with a correlated response in female body size. For male-biased SSD species male-male competition favours larger males with better competitive skills (Abouheif & Fairbairn, 1997; Fairbairn, 1997; Cox et al., 2003; Székely et al., 2004; Dale et al., 2007), whereas for species with female-biased SSD selection favours small males with better agility (Székely et al., 2004). Both scenarios lead to a SSD following a pattern of allometry consistent with Rensch's rule. In our model species, as in many newts, courtship, consisting of elaborated behavioural displays towards females and interactions between males, is key to ensure a successful insemination and the related fitness benefits. Male-male competition includes direct agonistic interactions and indirect sexual interferences during other males' courtship (Faria, 1995; Aragón, 2009a, b). Therefore, if sexual selection were favouring either larger or smaller males to improve direct or indirect interactions, we would expect a positive allometry for SSD consistent with Rensch's rule. However, we found an opposite pattern, suggesting that sexual selection acting on males is not playing a major role in the evolution of SSD in this species. Accordingly, a experimental study on *L. boscai* male courtship displays towards females showed that larger male body size is not associated with higher competitive ability (see Aragón, 2009b for details), which supports the lack of compliance with Rensch's rule. Furthermore, new analyses of the experimental data from Aragón (2009b) provided no relationship between female avoidance behaviour events during male courtships and male size (Appendix 2.4). Thus, published and new results do not support sexual selection on male size in *L. boscai*.

Stronger sexual selection in females may further explain the observed inverse pattern of allometry for SSD (Dale et al., 2007). Although sexual selection has been mainly explored in males, this evolutionary force can also be important in females. Sexual selection on females can emerge from competition for access to mates, breeding territories and other critical resources for reproduction, including food (Clutton-Brock, 2009; Rosvall, 2011). Previous research on *L. boscai* courtship behaviour found no relationship between female size and variables reflecting male interest in females, including the latency, and the number and duration of courtship behaviours (Aragón, 2009b). Thus, if sexual selection acting on female body size played a role in shaping part of SSD in *L. boscai* it is probably not mediated through female attractiveness. Additionally, female reproductive investment is highly energy-consuming and competition amongst females escalates as nutritional sources decrease and population density increases (Rosvall, 2011). In *L. boscai* reproductive effort is almost continuous throughout the year (García-París et al., 2004), and experimental evidence shows a strong competition amongst females in a foraging context (Aragón, 2009a). Moreover, only female body size is negatively related with conspecific density (Peñalver-Alcázar et al., 2017). Thus, part of the geographic variation in female size could be a costly consequence of natural selection via resource acquisition effects on female fecundity.

The fecundity selection hypothesis is commonly proposed to explain female-biased SSD and the inverse Rensch's rule (Hedrick & Temeles, 1989; Fairbairn, 1997), although criticism exist (Shine, 1988; Pincheira-Donoso & Hunt, 2017). Similar to other ectotherms, larger females often have larger clutches in many amphibians (Morrison & Hero, 2003). Intraspecific tests of Rensch's rule related inverse allometric patterns to an increased reproductive output in larger females in fishes (Herczeg et al., 2010) and anurans (Liao, 2013; Liao et al., 2015). Furthermore, some studies reported a strong correlation between female body size and clutch size in different newt species (Verrell & Francillon, 1986; Cvetković, Kalezić, Djorović & Džukić, 1996), showing that sex differences in body size increase accordingly with the fecundity hypothesis (Ficetola et al., 2010). However, analyses on the relationship between female body size and different parameters of fecundity in the Iberian newt do not support fecundity selection (Brea et al., 2007; this study). This may be explained by *L. boscai's* oviposition behaviour, which entails scattering their eggs both in space and time. Females do not lay their eggs in one clutch but individually wrap them in leaves, or attach them to the underside of rocks (Díaz-Paniagua, 1986; Orizaola & Brana, 2003). This behaviour leads to a long oviposition period that may last from three or four months up to the whole aquatic phase (Díaz-Paniagua, 1986; Brea et al., 2007). The temporal and spatial dispersion of the eggs may result in an increase in clutch size without an accompanying increase in female body size (Salthe & Mecham, 1974). The long oviposition period means that body size may not be as strong a selective force as in anurans, which usually have shorter breeding periods. Moreover, differences in environmental factors such as the length of the activity season and food availability across the geographical distribution of the species may play a major role in the interpopulational variation of fecundity (Pincheira-Donoso & Hunt, 2017). Interestingly, even if female body size and fecundity had evolved in concert, a positive selection on fecundity could not always be associated with female-biased SSD consistent with the inverse Rensch's rule (Monroe, South & Alonzo, 2015; Pincheira-Donoso & Hunt, 2017).

Our results on SSD can be partially explained by the differential plasticity hypothesis (Fairbairn, 2005), resulting from sex-specific plastic responses to food availability and intraspecific competition. Likewise, a previous study reported greater phenotypic plasticity in body size of *L. boscai* females compared to males. This was explained by evidence pointing to female, but not male, use of social information to obtain food resources. Females therefore engage in more agonistic interactions than males during feeding activities in a low resource environment, forcing females to assume higher costs derived from intraspecific competition (Aragón, 2009a; Peñalver-

Alcázar et al., 2017). The link between the current findings on SSD and our previous research is the inverse of Rensch's rule: female-biased SSD is positively correlated with female body size. This convergence in the proximal mechanism after controlling for the genetic component in both studies cannot be attributed to the idiosyncrasy of the database since the present study encompassed two years. Finally, additional sources of variation shaping SSD in this species may exist. In fact, our results also reveal a partial, but lesser, contribution of the genetic component from both nuclear and mitochondrial DNA markers, suggesting that several factors play contrasting roles at different temporal scales (Martínez-Solano et al., 2006; Freeland, Petersen & Kirk, 2011; Peñalver-Alcázar et al., 2017).

Overall, our two-year study suggests that resource availability and relative female density acting on female size are important drivers behind the pattern of allometry for SSD consistent with the inverse Rensch's rule in *L. boscai*. The observed pattern matches with earlier intraspecific research on female-biased SSD ectotherm species, and is compatible with the interface of the density-dependent resource availability hypothesis and the differential plasticity hypothesis. This contrast with the widespread view that female-biased SSD and thus the inverse Rensch's rule arise from strong fecundity selection favouring larger females. We propose alternative avenues related to competition amongst females for resources that ultimately can be linked with reproductive success. We emphasize the advantage of integrating results from experimental and biogeographical approaches to elucidate the mechanisms underlying geographical patterns at the intraspecific level.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the APPENDICES

Appendix 2.1 Field and literature-based population data
Appendix 2.2 Analyses excluding populations with low samples size
Appendix 2.3 Analyses using averages of SVL and SDI
Appendix 2.4 Courtship behaviour experiment in *Lissotriton boscai*
Appendix 2.5 Reproductive investment in *Lissotriton boscai*
Appendix 2.6 Spatial autocorrelation in PLSR analysis
Appendix 2.7 Model comparison for effects of ecological parameters on SDI

BIOSKETCH

Miguel Peñalver-Alcázar is interested in the study of patterns and processes along geographical and environmental gradients using amphibians and reptiles as model species. Pedro Galán is interested in the evolutionary biology of amphibians and reptiles, especially in aspects related to reproduction and population dynamics, as well as the conservation of threatened species. Pedro Aragón is a Ramón y Cajal researcher at the MNCN. He has broad interests in fundamental and applied ecology. He is currently interested in detecting underlying processes of spatio-temporal biodiversity patterns at different scales.

PA and MPA conceived the study and designed the field sampling. PA and PG collected experimental data. MPA collected field and literature data, analysed the data and led the writing of the manuscript. PA and PG discussed the results and contributed on the writing.

CHAPTER III

Niche differentiation between deeply divergent phylogenetic lineages of an endemic newt: implications for Species Distribution Models

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Under review

ABSTRACT

Species distribution models (SDMs) are used to address different questions in biogeography, conservation, ecology and evolution. A major drawback is that they usually treat a species as a single unit neglecting that a species responses to the environment may not be uniform across its distribution. Ecological differences between genetically differentiated populations may result in niche divergence for species with deeply geographical-structured and divergent intraspecific lineages. Here, we explore the role of intraspecific variation on the overall performance of SDMs and the ecological niche using an endemic Iberian amphibian (*Lissotriton boscai*) with two geographically highly structured lineages. For this, we built species and lineage distribution models using three different presence-only algorithms. We also tested for niche overlap, niche equivalency and niche similarity by means of an ordination technique. We found differences in the potential distribution of the two lineages and the underlying environmental factors. Moreover, an important contribution of this study is that intraspecific differences in the distribution model predictive capacity were detected independent of the inter-algorithm variability. This was coupled with lineages showing a very low degree of niche overlap and occurring in relatively different environmental niches spaces. The intraspecific variation observed in *L. boscai* led to an improved intraspecific predictivity of the lineage level based-distribution models. Finally, there was partial spatial agreement between the niche overlap and independently described secondary contact zones. This study highlights that SDMs at the species level only might be too naive to predict impacts of global change in species distributions.

Keywords: Species distribution models, environmental niche, intraspecific variation, intraspecific phylogenetic lineages, niche divergence, niche conservatism

INTRODUCTION

Where species occur and what factors drive and define their distributions and niches are aged and chief questions in biogeography, ecology and evolution. Species distribution models (SDMs), or ecological niche models (ENMs), correlate species occurrences records with climatic and/or environmental variables to derive predictions on species geographical distribution and habitat suitability (Franklin, 2010). The environmental-species relationships provided by SDMs allow researchers to tackle a diverse array of topics. For instance, risk assessment of climate change and invasive species on biodiversity (Araújo & Peterson, 2012), improve the sampling efforts for rare species (Guisan et al., 2006a), quantifying the environmental niche of species (Broennimann et al., 2012), and assessing evolutionary hypothesis (Wiens & Graham, 2005). The SDM-research bloom (Lobo, Jiménez-Valverde & Hortal, 2010) is matched with the increasing development of statistical tools, processing power and computational pipelines, biodiversity networks and databases, and environmental data (Graham, Ferrier, Huettman, Moritz & Peterson, 2004; Guisan et al., 2006b; Kozak, Graham & Wiens, 2008).

The use of SDMs is subject to several limitations similar to other statistical techniques, including the assumption that species distributions are at (quasi-) equilibrium with the environmental conditions within a given region/area, the inter-model variation in prediction performance, and the complexity of bridging the gap between the realized distribution and the potential distribution (e.g., Guisan & Thuiller, 2005; Jiménez-Valverde, Lobo & Hortal, 2008; Elith & Graham, 2009). One of the main shortcomings is that SDMs unrealistically assume the absence of intraspecific variation (phylogenetic and/or trait) within species. This leads to conclude that species populations respond and perform homogeneously to climatic and environmental conditions and/or changes across its distribution range and therefore assuming that the species niche is constant over space and/or time (Smith, Godsoe, Rodríguez-Sánchez, Wang & Warren, 2019). However, the profuse phylogeographic and evolutionary ecology research show that intraspecific variation is the very feature of natural populations (Moran, Hartig & Bell, 2016; Zamudio, Bell & Mason, 2016). Moreover, species may also show geographic/regional variation on their niches, "intraspecific geographic variation in niches" *sensu* Holt (2009), especially those with a marked phylogeographic structure (D'Amen, Zimmermann & Pearman, 2013). Therefore, acknowledging the intraspecific variation within species is a relevant challenge to improve the overall performance of SDMs (Zimmermann, Edwards, Graham, Pearman & Svenning, 2010). The studies that have so far addressed this question show its importance to improve, for instance, conservation strategies, risk assessment for invasive species and the knowledge of the species evolutionary history (e.g., Schulte et al., 2012; D'Amen et al., 2013; Maia-Carvalho et al., 2018).

Evolutionary biologists have long pursued to unveil the mechanisms underlying population differentiation and species formation. Geographic variation within species or species populations is the very source that feeds the development of ecological and evolutionary theories (Gould & Johnston, 1972). Intraspecific geographic variation research has commonly focused on exploring the variation of a set of morphological, behavioural, physiological characters and life-history traits of animals and plants along latitudinal, climatic and environmental clines (Lomolino, Sax, Riddle & Brown, 2006; Moran et al., 2016). However, recent studies have moved towards the study of geographical variation of an "abstract character" (Peterson & Holt, 2003), the ecological niche. Ecological differences within evolutionary lineages may foster divergent natural selection (niche divergence), promoting the evolution of reproductive isolation through adaptation to contrasting ecological niches (Schluter, 2009). This might occur in a sympatric setting (Via, 2001), but also in allopatric settings if diverging lineages inhabit geographical areas with contrasting environments (Kozak & Wiens, 2006). In the other hand, evolutionary lineages may retain ancestral ecological adaptations (niche conservatism) leading to an allopatric divergence under similar ecological conditions/niches. (Peterson, Soberón & Sánchez-Cordero, 1999; Wiens & Graham, 2005). Therefore, bridging different sources of evolutionary and ecological evidence may help to understand intraspecific differences within species and eventually benefit the species delineation. The increasing development of molecular, ecological modelling and statistical tools (Guisan et al., 2006b; Kozak et al., 2008) has made this possible by allowing a better phylogenetic-informed species distribution-modelling approach (e.g., Pearman, D'Amen, Graham, Thuiller & Zimmermann, 2010; Fitze et al., 2011).

In the present study we explore to what extent and why the assumption of non-intraspecific variation affects the overall quality of predictions derived from the SDMs, while minimizing other of its limitations (inter-model variability and niche equilibrium) to reduce potential noise. We selected as a model system the Iberian newt (*Lissotriton boscai*), an endemic newt to the Western half of the Iberian Peninsula comprising two major geographically structured lineages. The two lineages have a long independent evolutionary history that can be traced back to the Miocene (Martínez-Solano, Teixeira, Buckley & García-París, 2006; Teixeira et al., 2015). The whole distributional range of *L. boscai* falls within an area which served as glacial refugium in southern Europe, where species distributions were beyond the influence of previous glaciations and hence are expected to be closer to equilibrium with contemporary climate (e.g., Svenning & Skov, 2007). All these features render our species as a suitable ectothermic vertebrate model for the scope of this study.

For our purposes, we firstly estimated and characterized the potential distribution and niche of the Iberian newt, modelling either the species as a whole or its two main intraspecific phylogenetic lineages. Then, we used the species and/or lineage level based-distribution models to predict the distribution of each other lineage, assessing thus the potential existence of intraspecific niche similarities or differences. We also applied an ordination technique approach to test the potential existence of niche equivalency and/or niche similarity between the species intraspecific phylogenetic lineages, which should provide further insights on the species niche geographic variation. The integration of SDMs and an ordination technique should provide a feedback link to improve the interpretation of the results from both methodologies.

MATERIALS AND METHODS

Study species

Lissotriton boscai (Lataste, 1879) is a small-bodied newt endemic to the Western Iberian Peninsula, ranging from sea level to 1870 m a.s.l. (Fig. 13). Species preferred aquatic habitats span the two major climatic areas of the peninsula, the drier Mediterranean domain and the most humid Atlantic domain, as well as transition zones between both domains. Previous studies reported body size interpopulation differences throughout the species range (Díaz-Paniagua & Mateo, 1999; Peñalver-Alcázar et al., 2017). Furthermore, phylogenetic and phylogeographic analyses using mitochondrial and molecular markers revealed that populations belong to two well geographically–structured and deeply divergent intraspecific lineages (Martínez-Solano et al., 2006; Teixeira et al., 2015). Lineage A is widely distributed throughout western Spain and most of Portugal, whereas lineage B is restricted to central and south-western Portugal (Fig. 13). The initial split between the ancestral populations of both lineages potentially took place 9 MYA during the Miocene. Then, they probably evolved in multiple allopatric refugia during the Plio-Pleistocene, resulting in relatively high levels of genetic distance (range 6.05-7.47%). The geographical distribution of *L. boscai* lineages shows a parapatric pattern with secondary contact zones, and the two lineages are suggested to be independent evolutionary significant units (ESUs, Moritz, 1994) for management and conservation efforts (Martínez-Solano et al., 2006; Teixeira et al., 2015).

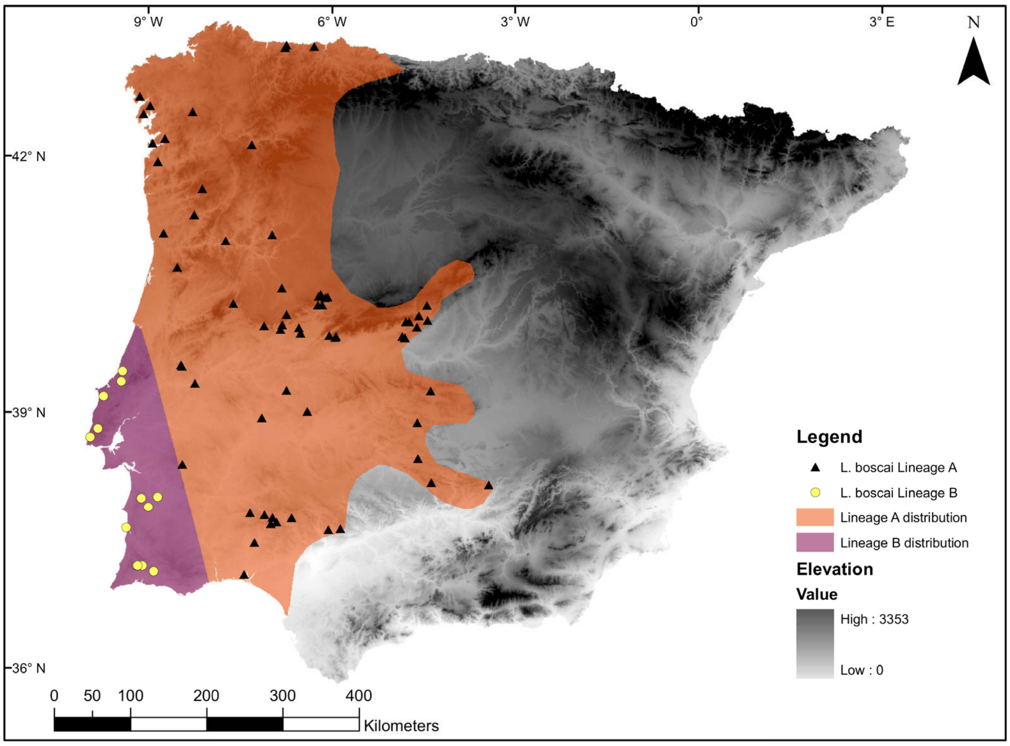

Figure 13 Presence points and distribution range of the *Lissotriton boscai* phylogenetic lineages A and B in the Iberian Peninsula. The presence records for lineage A are show as black triangles, presence records for lineage B are show as yellow circles. Distribution range for lineage A is coloured in orange, while distribution range for lineage B is coloured in purple (modified from Martínez-Solano et al. (2006). Background map shows the elevation, where higher intensity in the grey scale represents higher altitude.

Species occurrences and environmental predictors

Locality occurrence records were gathered from recent biogeographic and phylogeographic research on this species (Martínez-Solano et al., 2006; Sequeira, Silva-Ferreira & Lopes, 2012; Teixeira et al., 2015; Peñalver-Alcázar et al., 2017) and only those that have been unambiguously attributed to lineage A or B were used. Overall, we collected data from 79 populations comprising 66 populations from lineage A and 13 populations from lineage B. Nineteen bioclimatic variables representing annual trends, seasonality and extreme or limiting environmental factors and one elevation variable were retrieved from WorldClim database (http://www.worldclim.org/;

Hijmans, Cameron, Parra, Jones & Jarvis, 2005) at 1 km^2 resolution. Primary productivity variables (mean and seasonality) were also calculated for the period 2000-2015 using monthly maps of Enhanced Vegetation Index (EVI) at 1 km^2 resolution generated from MODIS satellite imagery available at https://search.earthdata.nasa.gov/search (Ruimy, Saugier & Dedieu, 1994; Peñalver-Alcázar et al., 2017). To prevent model overfitting and reduce multicollinearity in the original set of environmental and elevation variables, we performed pairwise correlation tests using the Pearson correlation coefficient following Box-Cox transformation. We selected a subset of variables that showed a Pearson correlation coefficient $< |0.8|$. For each pair of highly correlated variables we selected only the variable that is deemed biologically relevant based on the autoecology knowledge of the model species (Guisan & Thuiller, 2005; Austin, 2007). The availability of standing water and resources during seasonal activity has been previously reported relevant for newt species (Wielstra et al., 2013; Peñalver-Alcázar et al., 2017). Therefore, with these two criteria in mind, we selected nine predictors: bio3 = isothermality, bio4 = temperature seasonality, bio8 = mean temperature of wettest quarter, bio10 = mean temperature of warmest quarter, bio11 = mean temperature of coldest quarter, bio15 = precipitation seasonality, bio16 = precipitation of wettest quarter, EVI mean = mean of primary productivity and EVI seasonality = primary productivity seasonality.

Distribution models

We fitted distribution models for the selected environmental variables and three presence data subsets: 1) the combined presences from lineages A and B (SP); 2) the presences from lineage A (LA); 3) the presences from lineage B (LB). For this, three different presence-only algorithms were used: Bioclim (a climate-envelope based model), Environmental Niche Factor Analysis (ENFA) and Maximum Entropy Model (MaxEnt).

Bioclim defines a multi-dimensional rectilinear envelope enclosing all the records of the species in the environmental space defined by the selected variables (Busby, 1991; Booth, 2018). To predict species environmental suitability in any given location, Bioclim compares the values of the environmental predictors treated as a multiple one-tailed percentile distribution at an unknown location to the percentile distribution of the values from known location. Unknown locations with values of environmental predictors closer to the 50th percentile (i.e., the median) are considered the most suitable locations for the species and thus present the higher probability of occurrence regarding the predictors included in the model. Distribution tails are not distinguished, so the 10th percentile is treated equal to the 90th percentile, both having the same probability value. This percentiles treatment grounds in the niche theory, where species fundamental niche is partially arranged according to their environmental optimum in a bell shape fashion (Heikkinen & Mäkipää, 2010). In Bioclim, only the most relevant predictors provided by ENFA were used (see below).

ENFA is a multivariate approach similar to principal component analysis (PCA) that transforms several potential correlated environmental predictors into a reduced set of uncorrelated factors retaining most of the information. ENFA differentiates from PCA in that is based on the niche concepts of species marginality and specialization and the species distribution is taken into account for the building of factors (Hirzel, Hausser, Chessel & Perrin, 2002). The first factor maximizes the marginality, describing the ecological distance between the species optimum in any ecogeographical variable and the global mean of that variable in the study area. The factor values range between 0 and 1, with low values indicating that a species occurs in widespread conditions through the study area and high values indicating that a species occurs in conditions that are rare. The following factors maximize the specialization, describing the ratio of the ecological variance of the species in relation to the average habitat. This factor informs about how restricted the species niche is in relation to the study area, with higher values indicating that species occurs in a narrow range of conditions. Finally, the tolerance (1-specialization) indicates how tolerant a species may be regarding the predictors included in the model. Smaller tolerance values imply a more restricted species niche and thus higher specialization (Hirzel et al., 2002). In this study, the selected environmental variables were first normalized using the Box-Cox transformation. Then, factors explaining most of the variance were retained using the MacArthur´s broken stick criterion and used for habitat suitability (HS) map computation. HS maps were calculated using the medians algorithm. Finally, we assessed the predictor relative contribution by ranking all predictors with respect to their absolute maximum coefficient value across the most relevant ENFA factors and calculating four quartiles (e.g., Aragón, Lobo, Olalla-Tárraga & Rodríguez, 2010b). Predictors with values above the 25th quartile were considered predictors of higher relevance.

MaxEnt provides HS outputs by identifying the maximum entropy distribution of a set of species occurrences and a randomly selected background of pseudoabsences based on environmental variables (Phillips, Anderson & Schapire, 2006). We used Maxent's auto-features, the default regularization multiplier parameter, a maximum of 500 iterations, a convergence threshold of 0.00001 and 10000 background points. The logistic output was selected to generate the potential distribution of the species or its lineages. From MaxEnt's relative importance output for each predictor in the model, we also assessed predictors with higher or lower relevance according to the threshold of the 25th quartile used for ENFA.

Bioclim and MaxEnt were fitted using the corresponding functions from the R package *dismo* (Hijmans, Phillips, Leathwick & Elith, 2011). ENFA was performed in Biomapper 4.0 (Hirzel, Hauser & Perrin, 2007).

Distribution model evaluation

We evaluated the accuracy of the different modelling techniques using an intra and inter-model cross-evaluation approach. For the intra-model evaluations, we randomly split the two subsets of presence records (LA or LB) into five groups, leaving out four groups for model fitting (training data) and one group for model evaluation (test data). For the inter-model evaluations, we performed two types of evaluation: 1) the combined presence records (SP) were used as a training data, and presence records from either lineage A or B were used for model evaluation (test data); 2) presence records from one lineage (LA or LB) were used as a training data, and the presence records from the other linage (LB or LA) were used for model evaluation (test data).

The accuracy assessment was measured using four different and common indices: the true positive rate (TPR), the false negative rate (FNR), the area under the curve (AUC) from the receiver operating characteristic curve (ROC) and the continuous Boyce Index (CBI) using a moving window width of 10. TPR is the number of true positives / [number of true positives + number of false negatives]. FNR is the number of false negatives / [number of true positives + number of false negatives] (Fielding & Bell, 1997). The ROC curve is the relationship between the TPR (sensitivity) and the false positive rate. AUC values of 1 represent a perfect fit, while 0.5 corresponds to random attribution (Manel, Williams & Ormerod, 2001). The CBI is a modification from the original Boyce Index (Boyce, Vernier, Nielsen & Schmiegelow, 2002). It varies from -1 to 1 and shows a similar performance to AUC (Hirzel, Le Lay, Helfer, Randin & Guisan, 2006). To calculate TPR, FNR and AUC accuracy indices, the predictive results of ENFA, Bioclim and MaxEnt were used to compute a confusion matrix either for a range of cut-off values (0–1; ROC analysis) or for the minimum training presence (MTP; Phillips et al., 2006) threshold criteria (TPR and FNR calculation) to the corresponding presence test data and pseudo-absence data set (i.e., MaxEnt background points). TPR, FNR and AUC accuracy measures derived from this procedure should be interpreted with caution, as ENFA, Bioclim and MaxEnt modelling techniques do not use proper absence data (Jiménez-Valverde, 2012, 2014). Therefore, we also used the CBI, a method specifically developed for evaluation against presence-only observations (Hirzel et al., 2006).

Intra and inter-predictivity across lineages for different modelling techniques

We assessed the intra and inter-predictivity across lineage distribution models (i.e., transferability) to investigate potential differences in lineage niches. For this, we used model evaluation measures and continuous HS scores from distribution model predictive maps. In this context, a higher predictivity for models fitted and evaluated with presence records from the same lineage rather than from the other lineage (i.e., low transferability) would inform about potential niche differentiation (Peterson & Holt, 2003; Fitze et al., 2011).

We first tested for the existence of differences between the accuracy indices retrieved from the intra and inter-model cross evaluations using linear mixed models. Model accuracy indices (TPR, FNR, AUC and CBI) were used as dependent variables, type of evaluation as fixed factor and the distribution-modelling algorithm (i.e., Bioclim, ENFA and MaxEnt) as a random factor. The distribution-modelling algorithm was selected as a random factor to acknowledge that different modelling techniques may impact the resulting predictions. Type of evaluation comprised three transferability approaches: 1) distribution models built using SP presence records and evaluated using LA or LB presence records (SP to LA or to LB); 2) distribution models built using presence records from one lineage and evaluated within the same lineage (LA to LA and LB to LB); and 3) distribution models built using presence records from one lineage and evaluated towards the other lineage (LA to LB and LB to LA). In the second case, accuracy measures derived from each of the 5-fold intra-model cross evaluation were averaged to perform unbiased comparisons with the inter-model evaluations. We fitted two different linear mixed models for each accuracy index based on the lineage targeted for the intra and inter-model cross-evaluation. Post-hoc Tukey's HSD tests were performed when type of evaluation significance existed.

Secondly, the inter-predictability across lineages was further assessed using the continuous predictive maps derived from the different distribution modelling techniques. Here, the predictive result derived from each distributional presence records subset was used to predict the habitat suitability of LA and LB. Then, Wilcoxon rank sum test was performed to compare predicted HS between lineages, as the assumptions of normality and homoscedasticity were not met in all the cases.

Niche overlap, similarity and equivalency

We assessed the potential statically significant differences between the ecological niches of the two lineages following the environmental-PCA method proposed by Broennimann et al. (2012) and implemented in the R package *ecospat* (Di Cola et al., 2017). First, a PCA on the selected subset of environmental variables was computed and the resulting environmental space for the study area was gridded at resolution of 100 x 100 cells. Background environmental space was defined using environmental data extracted from 3000 random points (both 1000 and 5000 background points were also used and did not provide different results, Visger et al. (2016). Then, a kernel smoothed density function was applied to the occurrences of the two lineages plotted on the gridded environmental space, obtaining the density of occurrence. The observed niche overlap along the gradients of the environmental-PCA was estimated by calculating the Schoener's *D* index which ranges from 0 (no overlap) to 1 (complete overlap) (Warren, Glor & Turelli, 2008).

Then, we performed a statistical test for the niche similarity and niche equivalence hypotheses (Warren et al., 2008; Broennimann et al., 2012). The niche similarity test assesses whether the ecological niches of two entities (hereafter lineages) are more or less similar (niche conservatism *versus* niche divergence) than expected by chance. It compares the observed niche overlap (*D*) value between the realized niche of the two lineages to the 95th percentile of the expected distribution of *D* values constructed when the density of occurrence in one lineage is randomly shift 100 times while the density of occurrence of the other lineage remains constant. Then, this process is repeated for the other lineage. If the observed niche overlap value is significantly higher or lower than the 95th percentile of expected *D* values, the null hypothesis of random similarity is rejected.

The niche equivalence test assesses whether the ecological niche occupied by two lineages are identical. It compares the observed niche overlap value *D* between the two realized niches to the 95th percentile of the expected distribution of *D* overlapping index values constructed when all occurrences of the two lineages are pooled and randomly split into two datasets 100 times, maintaining the same number of occurrences as in the original lineages datasets. If the observed niche overlap value *D* falls outside of the 95th percentile of expected *D* values, the null hypothesis of random equivalency is rejected.

We also mapped within a geographical context (i.e., the Iberian Peninsula) the PCA scores for the two axes retrieved by the environmental-PCA. The aim was to geographically visualize the environmental gradients reported by the PCA axes while differentiating those values encompassed by the niche overlap between the two lineages. The latter was set up to explore if areas of niche overlap might inform on locations where secondary contact between lineages exist. As explained above, *ecospat* procedures use PCA scores to create a gridded environmental space for the occurrence densities of the lineages from which calculate and visualize niche overlap. Following this reasoning, PCA scores were first extracted for the georeferenced set of lineages presence records and background points used in *ecospat* procedures. Then, they were imported into a GIS environment, where PCA scores were interpolated to the extent of the Iberian Peninsula using an Inverse Distance Weight approach. Finally, the raster images provided by this procedure were reclassified to geographically delimit PCA score values where niche overlap was detected in Fig. 16 by the minimum rectangle that encompasses the overlap (see Fig. 16a). Final raster images comprised values from 0 (no overlap) to 1 (overlap).

RESULTS

Predictors' relevance for species and lineage distributions

MaxEnt revealed that *L. boscai* distribution in the Iberian Peninsula was best predicted by precipitation seasonality and mean primary productivity (Table 5). ENFA provided a marginality value of 0.70 and specialization value of 1.50. Precipitation seasonality and mean primary productivity contributed the most to the marginality axis (Table 5; bio15 and EVI mean = 0.51), while mean temperature of warmest quarter and mean temperature of coldest quarter contributed the most to the specialization axis (Table 5; bio10 = 0.75 and bio11 = 0.64, respectively). Predictive maps of area suitability for the two lineages and the three modelling techniques are shown in Supporting information Appendix 3.1, Fig. A3.1.

The most relevant variables for predicting *L. boscai* lineage A were also mean primary productivity and precipitation seasonality in MaxEnt (Table 5; EVI mean = 31.1% and bio15 = 29.9%). Marginality, specialization and tolerance values from ENFA were 0.60, 1.76 and 0.57, respectively. Mean primary productivity and precipitation seasonality defining marginality axis (Table 5; EVI mean = 0.56 and bio15 = 0.45) and mean temperature of wettest, warmest and coldest quarter defining specialization axis (Table 5; bio8 = 0.74, bio 10 = 0.67 and bio11 = 0.58).

Finally, *L. boscai* lineage B was best predicted by temperature seasonality and precipitation seasonality in MaxEnt (Table 5, bio 4 = 54% and bio15 = 33.2%). ENFA provided a marginality value of 1.75, specialization value of 5.44 and a tolerance value of 0.18. Temperature seasonality contributed the most to the marginality axis followed by isothermality (Table 5; bio4 = 0.59 and bio3 = 0.53). Specialization axis was mainly defined by precipitation seasonality (Table 5; bio15 = 0.80), followed by mean temperature of wettest quarter and mean primary productivity (Table 5, bio8 = 0.74 and EVI mean = 0.26). These higher marginality and specialization values for lineage B distribution, coupled with low tolerance values, indicate that the lineage's niche not only shows a high deviation from the mean values of the available conditions for the species, but also that is restricted to a narrow set of conditions, which is compatible with a high degree of specialization. Predictive maps retrieved by the different modelling techniques best depict this situation. They clearly show that lineage B have a narrow potential distribution, with higher habitat suitability values mainly restricted to central and south-western Portugal (Appendix 3.1, Fig. A.31).

Table 5 Relative contribution of the six most influential variables (67%) for predicting the distribution of *Lissotriton boscai*, considered as a whole species unit, and its two phylogenetic lineages using ENFA and MaxEnt. For ENFA, variables in bold denote variables with higher scores on the marginality factor than in specialization factors. Variables names correspond to: bio3 = isothermality, bio4 = temperature seasonality, bio8 = mean temperature of wettest quarter, bio10 = mean temperature of warmest quarter, bio11 = mean temperature of coldest quarter, bio15 = precipitation seasonality, bio16 = precipitation of wettest quarter, EVI mean = mean of primary productivity and EVI seasonality = primary productivity seasonality.

ENFA		MaxEnt	
Variable	Contribution	Variable	Contribution (%)
Lissotriton boscai			
bio10	0.75	bio15	36
bio11	0.64	EVI mean	32.8
bio4	0.52	bio8	7.1
bio15	**0.51**	bio10	6.6
EVI mean	**0.51**	bio4	6.1
bio16	**0.37**	bio16	4.5
Lissotriton boscai lineage A			
bio8	0.74	EVI mean	31.1
bio10	0.67	bio15	29.9
bio11	0.58	bio8	11.3
EVI mean	**0.56**	bio10	7.4
bio15	**0.45**	bio11	4.5
bio4	0.43	bio3	4.3
Lissotriton boscai lineage B			
bio15	0.80	bio4	54
bio8	0.74	bio15	33.2
bio4	**0.59**	EVI seasonality	4.7
bio3	**0.53**	bio16	4.2
bio16	**0.29**	bio11	2.1
EVI mean	0.26	bio10	0.9

Intra and inter-predictivity across lineages

Niche differentiation was assessed based on the potential existence of statistical differences between accuracy indices and HS scores across types of model evaluations and lineages using an intra and inter-predictivity approach. Linear mixed models of accuracy indices obtained when projecting distribution models to lineage A targeted for intra and inter cross-evaluations showed significant differences between evaluation types for all the accuracy indices ($P < 0.001$ in all cases; Fig. 14a, c, e and g). *Post-hoc* comparisons revealed higher TRP, AUC and CBI indices values and lower FNR index values when SP and LA models were used instead of LB models to predict LA (Tukey HSD test: $Padj < 0.001$ in all cases; Fig. 14a, c, e and g). Accuracy indices values did not significantly differed between SP to LA and LA to LA types of evaluation (Fig. 14a, c, e and g; Tukey HSD test: $Padj > 0.05$ in all cases).

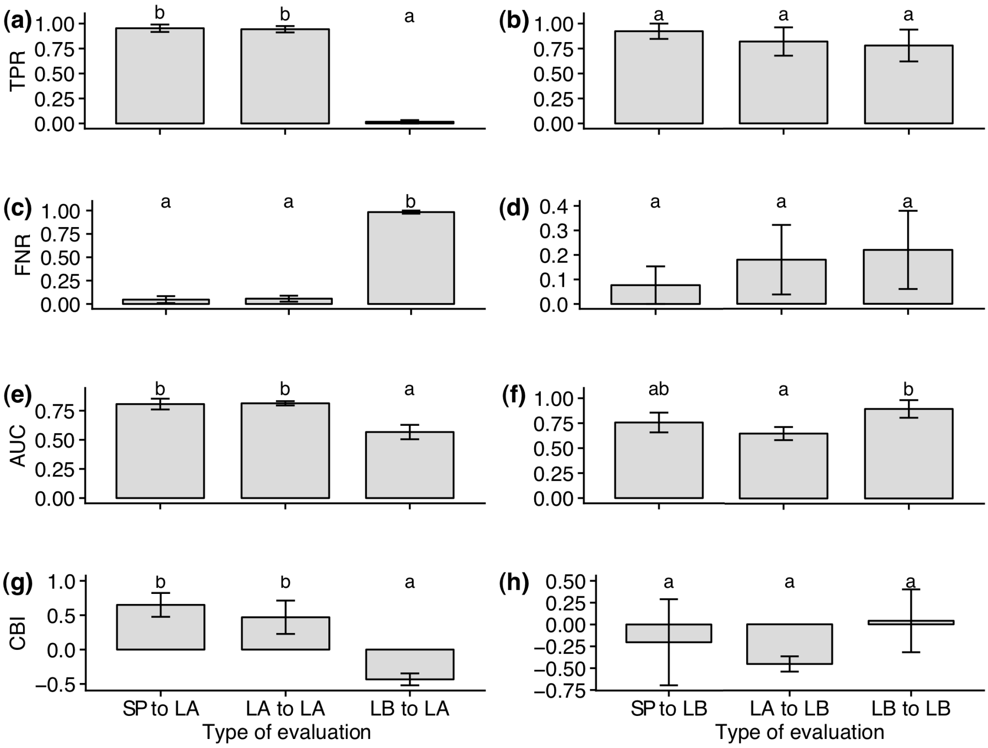

Figure 14 Mean ± SE of (**a-b**) true positive rate (TPR), (**c-d**) false negative rate (FNR), (**e-f**) Area Under the ROC curve (AUC) and (**g-h**) continuous Boyce index (CBI) values per evaluation type. Means denoted with different letters indicate significant differences between two evaluation types in *post-hoc* comparisons. SP to LA or LB denotes distribution models built using combined presence records and evaluated using presence records from lineage A or B, respectively. LA to LA and LB to LB denotes distribution models built using presence records from one lineage and evaluated within the same lineage. LA to LB and LB to LA denotes distribution models built using presence records from one lineage and evaluated towards the other lineage.

Linear mixed models of accuracy indices obtained when projecting distribution models to lineage B targeted for intra and inter cross-evaluations revealed non-significant differences between evaluation type for the TPR, FNR and CBI indices ($P > 0.05$ in all cases; Fig. 14b, d and h). They showed significant differences only for the AUC index ($P < 0.03$; Fig. 14f). *Post-hoc* comparisons revealed higher AUC values when lineage B was used instead of lineage A to predict itself (*Padj* < 0.024; Fig. 14f).

Regarding differences in HS scores across lineages, Wilcoxon rank sum tests provided mixed results when distribution models for combined presences of lineage A and B (SP) were used to predict one or another lineage.

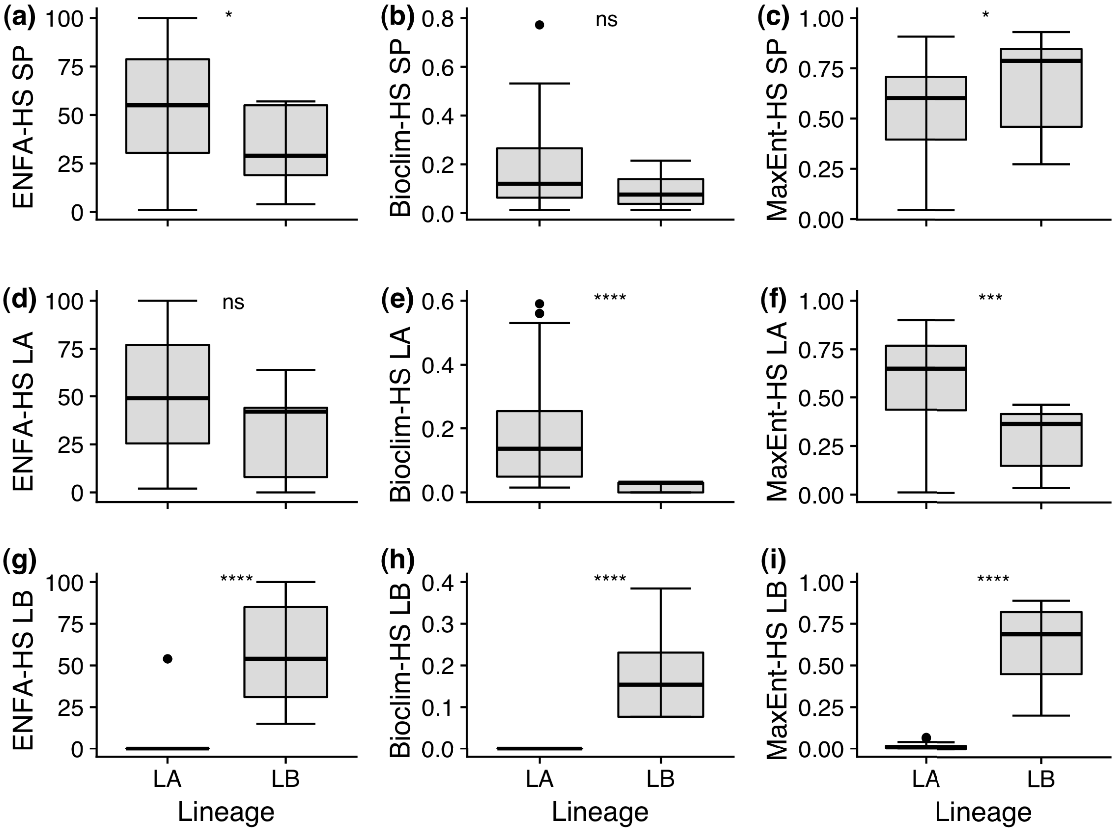

Figure 15 Comparison of habitat suitability (HS) scores predicted by ENFA, Bioclim and MaxEnt for the presence records from *Lissotriton boscai* lineage A (LA) and B (LB). (**a-c**) Boxplot of the HS scores derived from distribution models built with both lineages (SP), (**d-f**) with LA and (**g-i**) with LB. The significance of the Wilcoxon rank sum tests is shown: ns indicates no significance ($p > 0.05$) and asterisks indicate statistical significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

For instance, ENFA showed statically significant differences between lineages (Fig. 15a; $P = 0.027$), with higher HS scores for lineage A than lineage B. However, Bioclim did not reveal statically significant difference across lineages (Fig. 15b; $P = 0.13$) and MaxEnt provided that HS scores were statistically significantly higher in lineage B (Fig. 15c; $P = 0.011$). For distribution models fitted for lineage A and used to predict both lineages, results showed that HS scores were statistically significantly higher for lineage A than lineage B in Bioclim and MaxEnt (Fig. 15e and f; $P < 0.001$ in both cases) or near marginally significant higher in ENFA (Fig. 15d; $P = 0.064$). Finally, HS scores comparisons across lineages when lineage B distribution models were used to predict both lineages showed that the lineage B had always statistically significantly higher HS scores than in the predicted lineage A (Fig. 15g, h and i; $P < 0.001$ for ENFA, Bioclim and MaxEnt).

Characterization of the available environmental space

The two first axes retrieved by the environmental-PCA explained 62.5% of the total environmental variance of the Iberian Peninsula (Appendix 3.2, Fig. A3.2a). The first axis (PC1 = 32.1%) shows a strong positive relationship with temperature seasonality and a strong negative relationship with precipitation of wettest quarter and mean of primary productivity (Appendix 3.2, Fig. A3.2b). It informs on an edge-to-interior environmental gradient on the Iberian Peninsula. This gradient varies from an ocean-coast climate with a weak thermal oscillation and higher rainfall in the wettest quarter (i.e., winter), resulting in higher primary productivity, to a continental-interior climate with higher fluctuations in primary productivity and rainfall (Appendix 3.3, Fig. A3.3a). The second axis (PC2 = 30.4%) is strongly and negatively related to mean temperature of warmest quarter and mean temperature of coldest quarter with a weakly and positive relationship with mean of primary productivity and precipitation of wettest quarter (Appendix 3.2, Fig. A3.2c). It informs on a north-to-south thermal and rainfall gradient, splitting the Iberian Peninsula on two halves. Mild temperatures and high rainfall all year round define the first half, while the second half is characterized by hot summers and cold winters coupled with rainfall seasonality (Appendix 3.3, Fig. A3.3b).

Niche overlap and niche equivalency and similarity tests

The observed niche overlap between *L. boscai* lineages was $D = 0.036$. Lineage A niche is wider than lineage B niche (Fig. 16a). Lineage B niche is located at lower PC1 and PC2 values compared to lineage A. This informs about the association of lineage B to locations with low temperature seasonality and elevated rainfall in the wettest quarter as well as with mild temperatures during summer and winter (Fig. 16a and Appendix 3.3, Figs. A3.3a,b). The niche similarity test was not significant for any of the pairs of comparisons ($P > 0.05$ in all cases, Figs. 16b, c), so the null hypothesis of niche similarity could not be rejected. The niche equivalency test led to the rejection of the null hypothesis of equivalent niches ($P < 0.05$, Fig. 16d).

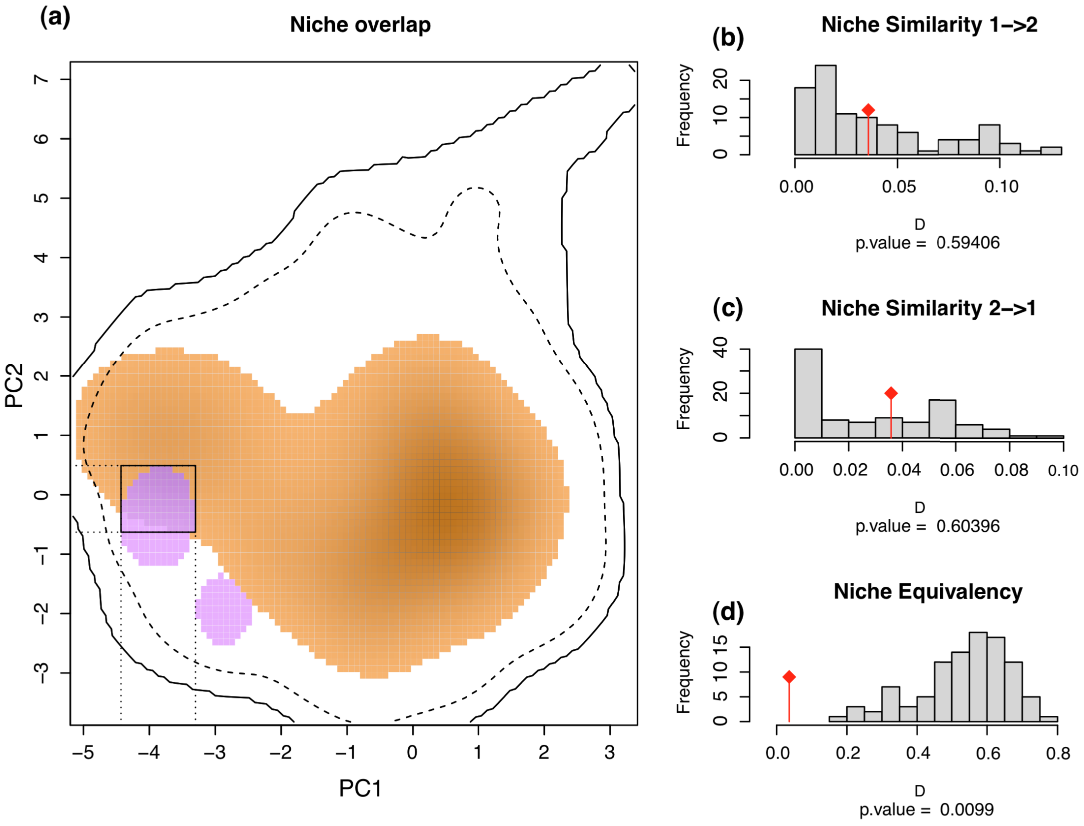

Figure 16 Niches of the *Lissotriton boscai* phylogenetic lineages in environmental space available in the Iberian Peninsula, and expected distribution of Schoener's *D* index values for the niche equivalence and niche similarity tests. Panel (**a**) represents the niche of lineage A (orange) and lineage B (purple) along the two first axes of the environmental-PCA as well as the overlap between the two niches. Niche overlap is delimited by the minimum rectangle that encompasses the overlap. Histograms (**b-d**) show the observed niche overlap *D* between the two lineages (bars with red diamond) and simulated niche overlaps (grey bars) on which tests of niche similarity lineage A to lineage B (**b**), niche similarity lineage B to lineage A (**c**), and niche equivalency (**d**) are based. The significance of the tests is shown.

Mapping the environmental-PCA axes scores bounded by the environmental overlap between both lineages within the geographical context of the Iberian Peninsula revealed two ecological niche overlap areas (Fig. 17 and Appendix 3.4). The first one is located in central-north Portugal and do not conform a continuous unit. It ranges approximately from the population of Valongo to the population of Nazaré (Fig. 17). The second area is restricted to the Lisbon Peninsula, encompassing the populations of Sintra and Aldeia da Mata (Fig. 17). Near these two areas, secondary contact zones between the two divergent lineages have been described in a previous study (locations indicated with a star in Fig. 17 from Teixeira et al., 2015).

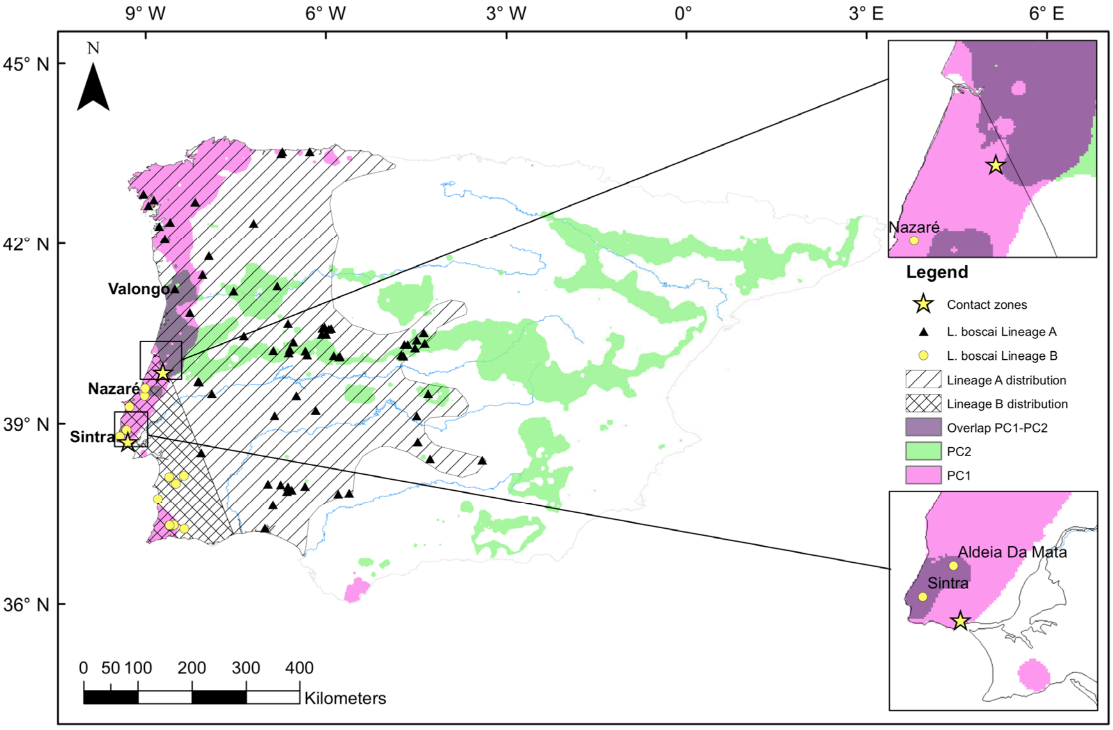

Figure 17 Geographical representation of the PCA scores reclassified using the values that delimit the area of niche overlap (rectangle in Fig. 16a) for the first two axes retrieved by the environmental-PCA mapped within the geographical context of the Iberian Peninsula. The presence records for lineage A are show as black triangles, while presence records for lineage B are show as yellow circles. Simple hatched represents distribution range for lineage A and cross hatched represents distribution range for lineage B. PC1 is coloured in pink, PC2 in green, and overlap between PC1 and PC2 in purple. Stars show localities where second contact between lineages A and B exist (see Fig. 1a in Teixeira et al., 2015). Niche overlap areas with contact zones are enlarged in separate panels.

DISCUSSION

SDMs often treat species as homogeneous units, thus neglecting the potential implications of the existence of intraspecific variation, with populations showing heterogeneous environmental requirements along the species distribution range. This could be even more relevant for species with a marked phylogeographic structure arising from deeply divergent intraspecific lineages (Pearman et al., 2010; D'Amen et al., 2013). Here, we address this issue in an endemic Iberian amphibian species (*Lissotriton boscai*) characterized by two main evolutionary lineages with a deep geographical structure. We integrated two different approaches: 1) testing the predictive capacity of distribution models across the two intraspecific lineages, and 2) using an ordination technique approach to characterize the niche overlap between the two lineages. Despite the inter-algorithm variability, we found intraspecific differences in the predictive capacity of distribution models as well as in the underlying environmental factors. This is compatible with the differences observed in the environmental niches between lineages, showing niche differentiation with a very limited degree of overlap.

The predicted potential distributions revealed that areas with higher suitability for either the species or its two intraspecific phylogenetic lineages are more likely to occur within the western half of the Iberian Peninsula and mostly similar to their actual known distribution, which it is bounded by the Guadalquivir river to the south-east, by Sierra Morena and Sierra de Guadarrama in Central Spain, and by Picos de Europa to the north (see Fig. 13 and Appendix 3.1, Fig. A3.1). The species potential occurrence matches with the common pattern of distribution of "true" Iberian herpetofauna endemic species such the anurans *Discoglossus galganoi* and *Rana iberica*, the urodele *Chioglosa lusitanica* and the squamata *Podarcis carbonelli* and *Lacerta schreiberi* (Borkin, 1999; Sillero, Brito, Skidmore & Toxopeus, 2009). Mean primary productivity and precipitation seasonality were the two most important predictors of the observed distribution of the species and its lineage A. Importantly, primary productivity (mean EVI) is relevant not only for the species distribution but also for the geographic distribution of a phenotypic trait of the species, the body size (Peñalver-Alcázar et al., 2017). This is compatible with previous results showing that SDMs might predict both the distribution and abundance of a salamander species and its phenotypic traits, which might help to explain mechanisms underlying biogeographical patterns (Lunghi et al., 2018). Temperature seasonality and precipitation seasonality were the most important predictors for lineage B, followed by mean temperature of the wettest quarter and mean temperature of warmest quarter. The dissimilarities between lineage A and B clearly respond to the extent of their distributions, which are concomitant with their environmental ranges. Lineage A distribution encompasses the bulk of the species distribution through mostly central, northwest and southwest Spain with a relatively higher suitability towards mainland areas, where a stronger Continental Mediterranean climatic influence is expected. Meanwhile, lineage B exhibits a narrow distribution with higher suitability near Atlantic coastal zones of central and south-western Portugal.

The observed differences between the two lineages in their pattern of distribution and suitable environmental conditions may reflect potential differences in their niches and ultimately in their responses to the available environment. Testing whether ecological characteristics of one lineage can successfully predict the geographic distribution of the other lineage (or itself) and *vice versa* provides a robust approach to address this issue (Peterson et al., 1999; Peterson & Holt, 2003). Indeed, our intra and inter-predictivity analyses resulted in statistical differences of accuracy indices and habitat suitability scores across types of model evaluations and lineages. Overall, we observed that distribution models built with the lineage B has a poor predictive capacity when projected on the lineage A, while lineage A performs relatively well when predicting lineage B. Moreover, projections of habitat suitability on each population revealed significant differences between lineages in almost every case, with the modelled lineage showing higher scores than the predicted lineage. These results are compatible with the existence of intraspecific niche differences in *L. boscai*. Despite the clear general trend of the above-explained intraspecific variability, we also found few cases in which results deviate from the general pattern in terms of direction or significance. This is in line with the well known uncertainty associated with the use of different modelling techniques at the species level (Acevedo, Jiménez-Valverde, Aragón & Niamir, 2016). Spatial predictions may be closer to the realized or the potential distribution depending on the nature of the modelling technique used (Jiménez-Valverde et al., 2008). Interestingly, we found that this uncertainty associated to the inter-algorithm variability was higher for models built at the species level (Fig. 15a-c) than at the lineage level (Fig. 15d-i).

Null model tests of niche overlap within the environmental space further inform on niche differences between *L. boscai* lineages. In this context, the observed niche overlap value points to a very limited or non-overlap between the two lineages (Rödder & Engler, 2011). Moreover, the rejection of niche equivalency hypothesis confirmed that the two lineages occur in different (not interchangeable or identical) environmental niches spaces. However the non-rejection of niche similarity hypothesis did not allow concluding that an outright niche divergence exists between the two lineages. Therefore niche differentiation between the two lineages cannot be strictly interpreted as a result of niche divergence, contrasting with the expectation derived from lineages with long independent evolutionary history (Peterson et al., 1999; Wiens & Graham, 2005). Such closely related but deeply divergent lineages in an allopatric setting are expected to evolve adaptations to local and contrasting environmental conditions and therefore more prone to exhibit niche divergence (Kozak & Wiens, 2006; Holt, 2009). Overall, the existence of niche differentiation coupled with a very limited overlap may arise from the heterogeneous environmental/ecological requirements/conditions available to each lineage within their distribution range (Warren et al., 2008). For instance, the more narrow environmental niche of lineage B seems to be related to Atlantic coastal zones with low temperature seasonality and elevated rainfall in the wettest quarter as well with mild temperature during summer and winter compared to the more mainland distributed lineage A. This pattern of niche differentiation is present in other Iberian amphibian and reptile species. Maia-Carvalho et al. (2018) found similar results for several of the seven allopatrically distributed intraspecific population lineages of the anuran *Alytes obstetricians* in the Iberian Peninsula. Specially, when comparing Atlantic and Continental climatic-influenced genetic clusters. Additionally, the modelling of four different and separate population subranges in the Iberian endemic lizard *P. carbonelli* revealed that inland subranges show a very limited or no niche overlap with those from Atlantic coastal subranges. Moreover, the inland subranges failed to accurately predict Atlantic coastal subranges and *vice versa* (Carretero & Sillero, 2016).

The geographic projection of the environmental niche overlap between the two lineages is compatible with an underlying process related to a hybrid zone arising from secondary contact. Secondary contact zones between lineages that have evolved in allopatry in different refugia are relevant to study evolutionary mechanisms related to lineage diversification, adaptation and speciation (Hewitt, 1988, 2001). We found that independent data on secondary contact zones (not included in model building) is closely located to the spatial projection of the environmental niche overlap between the two

L. boscai lineages. A potential explanation is that the environmental requirements shared by the two lineages are the common place where admixture across the two lineages can occur. This approach might prove useful for discovering, delineating and tailored sampling zones where allopatric lineages meet and exchange genes in other species, especially for those with a deep phylogeographic structure. Moreover, it highlights that bridging different sources of knowledge (i.e., historical and ecological biogeography, phylogenetic and phylogeographic approaches) helps to improve ecological and evolutionary understanding of the species (Wiens & Donoghue, 2004).

Addressing intraspecific variation in species distribution and niche modelling helps to better tackle relevant biogeographical and evolutionary questions (Kozak et al., 2008; Zimmermann et al., 2010; Gotelli & Stanton-Geddes, 2015), and studies combining climatic, environmental and phylogenetic information are gaining momentum in recent times (Smith et al., 2019). Compared to previous studies (e.g., Fitze et al., 2011; D'Amen et al., 2013; Maia-Carvalho et al., 2018), the main feature of our research entails the systematic test of the predictive accuracy of different SDM algorithms (Bioclim, ENFA and MaxEnt) and simultaneously collating it with an *ad hoc* ordination method to quantify intraspecific niche differences (*ecospat*). Additionally, exploring the potential match of the environmental niche overlap with secondary contact zones may aid to delve into the ecological and evolutionary understanding of the species.

The common practice of SDMs relies mostly on a species level approach that underestimated the existence of intraspecific differences. In most cases, species level based-SDMs show a reduced forecasting capacity compared to those lineage-based models (above or below the species level). Therefore, SDMs constructed at the species level and projected in space and/or time within an applied ecology framework (e.g., climate change or invasive species) could have even greater associated uncertainty than previously acknowledged. This uncertainty may arise from processes at the intraspecific level, such as phylogenetic inertia and/or differential adaptation. The present study takes a step further in this emerging "topic" since that the intraspecific processes-related uncertainty assumption can only be reached considering different modelling algorithms, as the objective of this study does not elude from the well-known inter-algorithm variability in terms of predictive performance. In other words, as far as possible, this type of studies should consider all sources of uncertainty since they can act together.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the APPENDICES

Appendix 3.1 Predictive maps derived from Species Distribution Models

Appendix 3.2 Principal Component Analysis (PCA) on the environmental variables related to the distribution of *Lissotriton boscai* phylogenetic lineages

Appendix 3.3 Environmental gradients of the Iberian Peninsula

Appendix 3.4 Mapping PCA scores for the two first axes from the env-PCA

•GENERAL

DISCUSSION•

GENERAL DISCUSSION

The study of intraspecific variation, or variation within species or between species populations, in space and time is at the core of biogeographical, ecological and evolutionary research (Gould & Johnston, 1972; Thorpe, 1987). Early naturalist, biogeographers and evolutionary biologist such as Alexander Von Humboldt, Karl Bergmann, Alfred Russel Wallace and Charles Darwin clearly understood the importance of this phenomenon. Today there is an increasing consensus on the urgent need to improve the knowledge and understanding of intraspecific variation due to its chief implications for the ecological communities and ecosystems functioning (Bolnick et al., 2011; Des Roches et al., 2018). Moreover, the study of the causes and mechanisms driving intraspecific variation is crucial in the context of global change and the rapid and increasing biodiversity loss (Bálint et al., 2011; Moran, Hartig & Bell, 2016), with recent research urging that conservation efforts should target intraspecific variation in the face of climate change, land use change, pollution and species introductions (Mimura et al., 2016; Raffard, Cucherousset, Santoul, Di Gesu & Blanchet, 2018).

The broad aim of this thesis was to provide new, robust and integrated insight into the different and complex factors and mechanisms underlying intraspecific variation among populations by studying patterns and processes related to the geographical variation of body size, sexual size dimorphism and environmental niche in our model species the Iberian newt, *Lissotriton boscai*. Overall, the results of this thesis allowed us to establish that the intraspecific variation among the studied Iberian newt populations (body size, sexual dimorphism and environmental niche) depends on sex and lineage-specific effects of environmental conditions mediated by different and non-exclusive mechanisms. Variables related to primary production, conspecific density and intraspecific competition, as well as seasonality, play an important role in the geographical variation of body size and sexual size dimorphism (SSD) (**Chapters I and II**). Moreover, female Iberian newts drive allometry in SSD (**Chapter II**). This leads us to conclude that the density-dependent resource availability hypothesis and the differential plasticity hypothesis explain the geographical variation in body size and SSD (**Chapters I and II**). Finally, primary production and its seasonality, along with temperature and precipitation seasonality, largely determine the differences between lineages in their geographical distribution and environmental niches, which are not interchangeable (**Chapter III**).

The biogeographical study of variation among populations first requires recognizing and describing the existence of patterns and then unveiling the processes behind them. However biogeographical patterns are governed by a complex set of interdependencies between the species and their environment throughout evolutionary-time scales. It is also widely recognized that these patterns arise from several factors and mechanisms that may act together, simultaneously or separately, at different spatial and temporal scales (Mayr, 1956; Lawton, 1999; Ricklefs, 2012). The best approach to tackle this challenge is integrating different sources of information at different spatio-temporal ecological and evolutionary scales with multiple hypotheses. This will ultimately lead to improving the knowledge of the ecology and evolution of biodiversity, in general, and the species in particular. In this thesis, we embrace such an integrated approach by combining climatic and ecological data at different spatial scales (from local to regional scales) with phylogenetic information at different temporal scales (from historical to contemporary evolutionary events) and link the data to multiple and alternative non-exclusive hypotheses.

The detailed study of geographical variation of body size (**Chapter I**) and SSD (**Chapter II**) has revealed that mechanisms related to primary productivity and intraspecific competition are driving the observed patterns, through different influences on female and male Iberian newts. Moreover, the finding of an allometric relationship between male and female body size consistent with the inverse of Rensch's rule suggest that body size divergence in females is greater than in males, which ultimately may have led to the existence of female-biased SSD in this species.

Previous studies have found that females and males can respond differently to climatic, ecological and social factors (e.g., Shine, 1989; Kvarnemo, 1997; Aragón & Fitze, 2014; Peñalver-Alcázar, Aragón, Breedveld & Fitze, 2016; Peeters et al., 2017). Moreover, females and males usually differ in their morphological characteristics and life-history traits because of their contrasting reproductive roles (Fairbairn, 1997). Females often compete more intensively among themselves for resources necessary for successful reproduction (e.g., to produce and rear offspring) such as food or breeding sites, while males often compete intensively among themselves for access to females and matting opportunities (Clutton-Brock, 2007, 2009). Therefore, the existence of sex-dependent selective mechanisms leading to body size variation and the evolution of SSD in *L. boscai* is not unexpected. Likewise, experimental evidence shows that male and female Iberian newts respond differently to the presence of conspecifics, due to their effect on access to food. Females compete intensively among themselves for access to food, while males usually compete intensely among themselves during courtship but not when foraging together (Faria, 1993, 1995; Aragón, 2009a, b). This had led to sex-dependent foraging strategies and efficiency. Moreover, female-female competition for food in amphibians is density-dependent. This density-dependent effect may increase or decrease based on the resource abundance (Kuzmin, 1995).

In this sense, we have found that females are more sensible than males to the combined influences of food availability and intraspecific competition derived from conspecific density. Therefore, *L. boscai* female body size variation results from differences in foraging efficiency, depending on the degree of female conspecific competition and the abundance and levels of food availability. Additionally, temporal variability in levels of food availability may had stronger influence on *L. boscai* male body size compared to females, due to the earlier arrive of males to the breeding sites (Faria, 1995; Caetano & LeClair, 1999). Our results are in line with other studies in amphibians that found that density-dependent competition interference for food drive body size variation over temporal and spatial scales (Green & Middleton, 2013; Ousterhout, Anderson, Drake, Peterman & Semlitsch, 2015). This supports the density-resource hypothesis and the differential plasticity hypothesis, leading to establish that resource limitation coupled with population density is driving geographical variation of body size and sexual size dimorphism.

Species distribution models (SDMs) have become standard tools to address a plethora of applied and fundamental questions in biogeography, ecology, evolution and conservation. Their widespread use has been matched with an increasing sophistication of the algorithms and methods employed. Nevertheless, one of the major drawbacks of SDMs practice is failing to account for the phylogenetic structure and relationships within a species' populations. This is of paramount importance, as divergent populations or intraspecific lineages (i.e., subspecies or evolutionary significant units) may exhibit geographical variation in their environmental requirements and niche.

In **Chapter III**, we addressed this issue by building lineage-specific distribution models. We found that the two evolutionary lineages of *L.* boscai have different and well-defined geographical distributions. While lineage A is distributed over a large part of the species distribution range in the western half of the Iberian Peninsula, lineage B has a smaller distribution, which is restricted to the southwest of Portugal. Phylogenetic lineages occupying different and structured geographical spaces can potentially also be influenced by different environmental requirements (Pearman, D'Amen, Graham, Thuiller & Zimmermann, 2010; D'Amen, Zimmermann & Pearman, 2013). In fact, we found that mean primary productivity and precipitation seasonality were the most relevant variables for lineage A distribution, whereas temperature seasonality and precipitation seasonality were among the most relevant variables for lineage B. Interestingly, these results highlighted that macroscale variables related to primary production and seasonality are not only important for body size, but also for the species and/or lineage distributions. The observed lineage-specific geographic distribution and environmental requirements resulted in a different predictive capacity and transferability of distribution models across the two lineages. While lineage A distribution models had good ability to predict the distribution of lineage B, lineage B distribution models showed a reduced ability to predict the distribution of lineage A. These differences are most likely related to the low degree of niche overlap observed between the two lineages that ultimately led to establish the existence of non-equivalent environmental niches.

The differences found in the geographical distribution and the ecological niche between the two lineages and, therefore, in the predictive capacity and transferability of the distribution models, are in line with previous studies on species with divergent lineages from the Iberian Peninsula. For instance, similar results have been observed for species of lizards (*Psammodromus hispanicus*; Fitze et al., 2011), amphibians (*Alytes obstreticans*; Maia-Carvalho et al., 2018) and water beetles (*Ochthebius glaber*; Sánchez-Fernández, Lobo, Abellán & Millán, 2011). However, the main differences with these studies lies in our systematic evaluation of the intra and inter-predictability between lineages, using different species distribution algorithms coupled with the quantification of the environmental niche by means of an ordination technique. At the same time, another surprising and contrasting difference with the aforementioned studies is that the niche differentiation observed in *L. boscai* lineages can not be attributed strictly to the existence of niche divergence. This result contrasts with the long independent evolutionary history of the two lineages, and with the expectation derived from lineages with long independent evolutionary history (Peterson, Soberón & Sánchez-Cordero, 1999; Wiens & Graham, 2005). Failing to find niche divergence may potentially be related to the sample size of lineage B (n = 13), which could be small to provide sufficient statistical power to establish an inference about niche evolution (Warren, Glor & Turelli, 2008). However, Warren et al. (2008) suggested that sample sizes below 5 are too small to find significant result, and that of lineage B is 160% above this threshold. Anyway, since this threshold might depend on the species' idiosyncrasies, future studies should focus on increasing the sample size in order to enhance representation of lineage B populations.

One of the main generalities emerging from the study of the biogeography of the Iberian newt is that primary production, as well as seasonality —primary production, temperature and precipitation— are the main factors related to the sex-dependent body size variation, and therefore of the existence of sexual dimorphism, as well as to the distribution of the species and its lineages.

Primary production has been proposed as one of the chief factors driving body size variation (both at inter and intraspecific levels) and species distribution, from mammals and birds to reptiles and amphibians (Hawkins et al., 2003; Rodríguez, Belmontes & Hawkins, 2005; Meiri, Yom-Tov & Geffen, 2007; Ficetola et al., 2010; Huston & Wolverton, 2011). In amphibians, productivity and seasonality seem to be closely related to the distribution patterns of species richness (Gouveia, Hortal, Cassemiro, Rangel & Diniz-Filho, 2013). The availability of food resources is fundamental to meet the energy and nutritional demands of animals during their development, growth and reproduction (Morrison & Hero, 2003; Huston & Wolverton, 2011). Furthermore, food availability for each animal/species is determined not only by the quantity and seasonal variability of the primary production in each region, but also by the population density (i.e., competition for food). Therefore, it is not uncommon that the intraspecific variation in body size is largely determined by the availability of resources per animal based on the degree of productivity and competition (McNab, 2010; Huston & Wolverton, 2011; Green & Middleton, 2013).

Similarly, temperature and water availability are key factors related to body size clines and patterns of distribution in amphibians (Buckley & Jetz, 2007; Aragón, Lobo, Olalla-Tárraga & Rodríguez, 2010b; Carvalho, Brito, Crespo & Possingham, 2011; Gouveia & Correia, 2016). Temperature and water affect all the facets of amphibians' biology (Wells, 2010). Amphibians rely on the external heat interchange with air and/or water to control their body temperature and set up in motion their physiology, behaviour and activity patterns. Temperature also determines the rate of development and growth, in eggs and larvae as well as in adults, the emergence for hibernation (or aestivation) and the start of reproduction. Water availability in breeding habitats (ponds, troughs, streams, etc.) is crucial for reproductive success, subsequent development of eggs and larvae and long-term survival of juveniles and adults. Additionally, amphibians need to balance keeping their skin moist to avoid dehydration (and favour gas interchange) with controlling their body temperature; so a complex physiological trade-off between temperature and water availability exist for amphibians. For all these reasons, water is one of the most crucial parameter for amphibians' distribution, especially in regions with contrasting temperature and precipitation regimes, such as the Iberian Peninsula (Borkin, 1999; Aragón et al., 2010b; Carvalho et al., 2011). The Iberian Peninsula exhibits a marked spatial and temporal climatic heterogeneity as a result of its position between the Atlantic Ocean and the Mediterranean Sea, with a seasonal north-south gradient of precipitation and temperature and a seasonal west-east gradient of precipitation (Rivas-Martínez, 2007). Therefore, fluctuations in precipitation and temperature, along with periods of drought, may affect not only amphibians' distribution in the Iberian Peninsula, but also relevant larval and adult morphological and life-history traits between populations along altitudinal or latitudinal seasonal climatic gradients. For example, larval life-history traits may show plastic or genetic adaptive genetic differentiation, favouring faster larval development and growth to face seasonal constraints but often resulting in a smaller size at metamorphosis and adulthood (Miaud & Merilä, 2001; Laugen, Laurila, Räsänen & Merilä, 2003; Gomez-Mestre, Kulkarni & Buchholz, 2013; Luquet, Lena, Miaud & Plenet, 2015).

Recognizing the existence of intraspecific variation, as in this thesis, is a relevant challenge for the study of patterns and processes in biogeography, ecology and evolution, and it will undoubtedly improve the species basic knowledge. Moreover, it is a key issue if we are to obtain better-informed predictions for understanding species responses to the current global change, that will ultimately contribute to better conservation strategies. For instance, the knowledge that females and males may respond differently to environmental factors such as food resources may favour sex-specific conservation measures. By doing so, conservation planning could focus on protecting or enhancing habitat features or land uses favourable to a given sex (van Toor, Jaberg & Safi, 2011). On the other hand, recognizing that divergent populations or lineages do not respond uniformly to environmental conditions throughout the species' distribution helps to further understand their environmental preferences and niche evolution. Compared to the commonly adopted species-level approach, the intraspecific approach thus provides crucial insight for conservation-decision making in the face of climate change, land use change, pollution and species introductions (Pearman et al., 2010; D’Amen et al., 2013; Smith, Godsoe, Rodríguez-Sánchez, Wang & Warren, 2019). Moreover, information on lineage and/or population-specific ecological requirements may complement other sources of evolutionary and ecological evidence, helping to resolve taxonomic uncertainties and to delineate species or evolutionary significant units (Wiens & Graham, 2005; Sánchez-Fernández et al., 2011).

In general, the shift of basic and applied conservation research and practice from its traditional focus on species to a focus on intraspecific variation is a promising avenue. This approach may shed light on relationships that are neglected or go unnoticed when the species is considered as a whole. Shifting from one approach to another requires caution and careful consideration of the pros and cons. Every species is ecologically and evolutionary unique, playing a specific role in a fine-tuned and dynamic ecosystem. Therefore, each species deserves attention and needs to be conserved. However the already limited conservation resources may eventually be further thinned down if conservation strategies are atomized, i.e., become sex or population-specific. Therefore, cost-effective conservation management plans and strategies should be based on a thorough intraspecific understanding of the targeted species along its distributional range, including a detailed autoecological knowledge of the species (behaviour, physiology, reproduction, etc.), its ecological requirements, location-specific knowledge on whether it is rare or common, its ecological and evolutionary significance, its resilience and resistance, its relationship with other species, etc. (Crandall, Bininda-Emonds, Mace & Wayne, 2000; Lindenmayer & Hunter, 2010; van Toor et al., 2011; Des Roches et al., 2018; Walls, 2018). This will help to create an integrated and holistic conservation framework.

Finally, the findings in this thesis allow us to establish future prospects to further improve the ecological and evolutionary understanding of the species and the mechanisms underlying biogeographical patterns, in general. For instance, the design and development of experiments with both field and lab-reared individuals might help to further elucidate the relevance of environmental and ecological factors, such as food availability, conspecific density and temperature, on body size variation. These experiments would also take into account phylogenetic factors, i.e., the lineage or clade (mtDNA and/or nDNA) of the populations to which individuals belong. In addition, heritability of body size might be estimated through a combination of common garden conditions (Turesson, 1922) for individuals from different representative populations with the experimental manipulation of the rearing conditions of the offspring born in the laboratory. This experimental setting could allow us to tell apart local adaptation from phenotypic plasticity and/or phylogenetic inertia (Conover & Schultz, 1995; Blanquart, Kaltz, Nuismer & Gandon, 2013).

One specific experiment might involve manipulating food availability and conspecific density. The experimental setting would consist of a factorial design combining the two factors, each one with two levels: high and low food availability/high and low conspecific density. This experiment could be performed on both adults (males and females) and larvae. The implemented levels of food availability and conspecific density would be determined based on the natural range of primary production and relative abundance throughout the distribution of the species. Another experiment could involve temperature. Temperature affects growth rate and body size in ectotherm species, with individuals reared at lower temperatures growing to a larger body size (Angilletta & Dunham, 2003; Angilletta, Steury & Sears, 2004). In the present thesis, we found that temperature was also relevant for body size variation, although to a lesser extent than food availability and conspecific density. To further explore the potential effect of temperature on body size, we would manipulate rearing temperature of eggs from females captured in the field and housed in the lab. Rearing temperatures would span the thermal range of the species (12, 17 and 22 ºC; Brea, Galán, Ferreiro & Serantes, 2007)). This experimental design would take into account the phylogeny (lineage/clade of both parents) and the body size of the mother. A similar experimental design could be carried out with food availability.

Performing the above-mentioned potential experiments over the course of the thesis was not feasible for logistical, economic and timing reasons. Since 2013, the transposition of the Directive 2010/63/EU for the protection and welfare of animals used for scientific purposes through the Real Decreto 53/2013, requires that vertebrate animals used for experimentation are housed in specific-designed facilities suited to the target species. Additionally, the relevant authorities in animal welfare must officially recognize the facilities, equipment and personal for animal experimentation. During the course of this thesis, although we obtained the certified recognition of competences for animal experimentation (Category B and C), there was no infrastructure for a specific-housing and experimental facility for amphibians in the Museo Nacional de Ciencias Naturales that was officially recognised by the relevant authority. In addition, it is worth mentioning that gathering enough individuals from all *L. boscai* populations throughout the species geographical range would be a challenging, complex and time-consuming task that eludes the majority of biogeographic studies on spatial and temporal patterns. In this context, we share James H. Brown thoughts about this issue in his book titled Macroecology (Brown, 1995): “It is often impractical, impossible, or immoral to perform replicated, controlled experiments on the spatial and temporal scales required to answer many basic and applied questions. Consequently, it is necessary to find alternative ways to make inferences about the natural world”. In any case, the experimental approach would complement, but not replace, the observational approach used in this thesis. Experimental manipulation may allow establishing cause-effect relationships by means of the comprehensive control of a specific array of factors. Nevertheless this may provide a reductionist view compared with the holistic view of the observational approach, since the former is not often able to grasp the complexity of nature. The two approaches have their strengths and weaknesses and their successful integration would be beneficial to improve the understanding of patterns and processes in biogeography (Lawton, 1996).

•CONCLUSIONS•

CONCLUSIONS

1. The pattern of body size variation among *Lissotriton boscai* populations is related to sex-depended responses to climatic and ecological factors after accounting for the (phylo)genetic structure of populations.

2. Individuals in populations with higher primary productivity and lower annual temperature, or smaller fluctuations in primary productivity, present larger body sizes. Local conspecific density was negatively related to body size in males and females, but was the ecological factor with the strongest sex-bias. For females this predictor was among the three of greatest relevance, whereas for males it was among the three least important predictors.

3. Female body size variation is mainly related to a process involving food availability, conspecific density and competition. This is in line with the hypothesis of density-dependent resource availability. In males, seasonal fluctuations in food availability was one of the most relevant predictors of body size variation, and several alternative non-exclusive hypotheses potentially underlie the pattern, namely: the heat conservation hypothesis, the temperature-size rule hypothesis and/or the seasonality hypothesis.

4. Interpopulation variation of sexual size dimorphism (SSD) increased with increasing female mean body size, supporting the existence of the inverse Rensch's rule pattern in *L. boscai*.

5. Primary productivity-related variables and female density were among the most relevant ecological predictors of SSD after accounting for genetic structure and temporal autocorrelation. The interface between the density-dependent resource availability hypothesis and the differential plasticity hypothesis explains the inverse of Rensch's rule.

6. The two intraspecific phylogenetic lineages of *L. boscai* differ in their potential distribution and underlying environmental factors.

7. Intraspecific differences in predictive capacity and transferability of distribution models were detected independent of the inter-algorithm variability. The

consideration of the intraspecific variation observed in *L. boscai* led to an improved intraspecific predictability of the lineage level based-distribution models.

8. *L. boscai* lineages showed a very low degree of niche overlap and occurred in relatively different environmental niches spaces (rejection of the null hypothesis of niche equivalence test). The existence of niche divergence or niche conservation could not be fully supported due to the non-rejection of the null hypothesis of niche identity test. There was partial spatial agreement between the environmental niche overlap and independently described geographical secondary contact zones between the lineages and/or clades of *L. boscai*.

9. Overall, primary productivity and seasonality of primary productivity, temperature and precipitation are among the most important macroscale predictors related to both interpopulational variation in body size, and hence to SSD, and the species and lineage geographical distributions and environmental niche.

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•APPENDICES•

APPENDIX I

SUPPORTING INFORMATION

CHAPTER I

Sex-dependent implications of primary productivity and conspecific density on geographic body size variation in a newt: disentangling local, large scale and genetic factors

Peñalver-Alcázar, M., Martínez-Solano, I., Sequeira, F. and Aragón, P. 2017. Journal of Biogeography 44: 2096-2108.

Appendix 1.1 Spatial autocorrelation in models

Spatial autocorrelation is an inherent property of environmental variables, and it can be defined as the lack of independence between two sets of observations with a given distance in space (Legendre, 1993; Borcard, Legendre, Avois-Jacquet & Tuomisto, 2004). The existence of spatial autocorrelation in the data can alter the results of statistical models, especially biasing and/or inverting parameter estimates and/or overestimating the contribution of environmental factors (Legendre et al., 2002). Spatial autocorrelation was explored by calculating Moran's *I* coefficient (significance evaluation by 199 permutations and sequential Bonferroni correction) based on the residuals from the final combined models and thereafter generating spatial correlograms (see Fig. A1.1) using SAM (Rangel, Diniz-Filho & Bini, 2010).

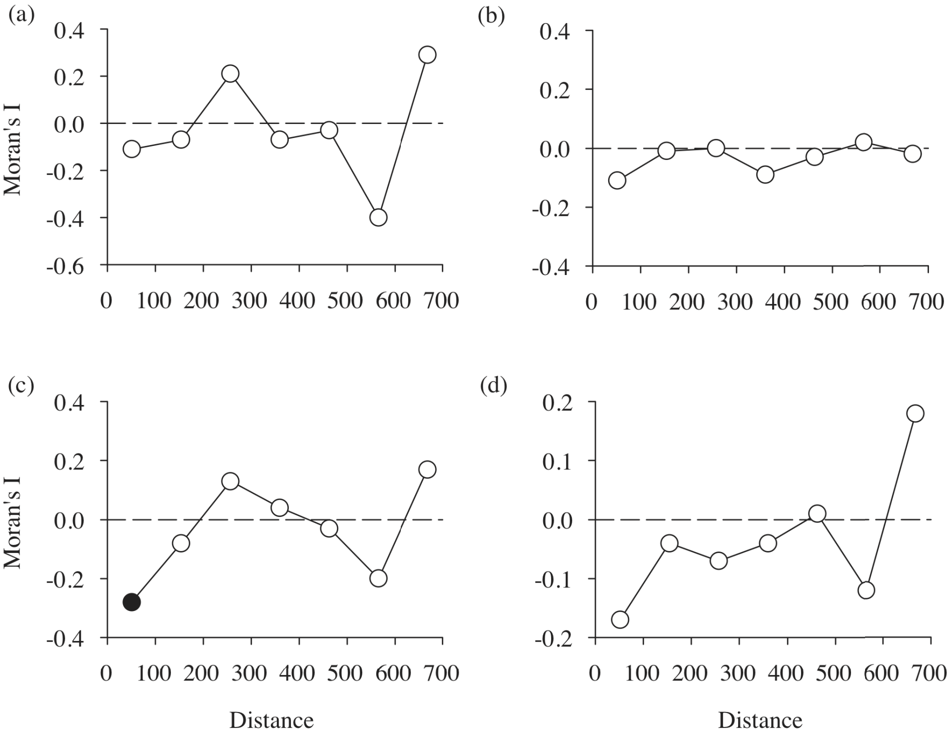

Figure A1.1 Spatial correlograms on final combined models (ecological + PVs predictors) residuals for female first (**a**), second (**b**) and third (**c**) relevant partial least squares regression (PLSR) component; and male first (**d**) relevant PLSR component. Open circles indicate no-significant correlation and solid circles indicate negative correlations after sequential Bonferroni corrections.

Appendix 1.2 mtDNA marker sequencing and phylogenetic analyses

We amplified and sequenced the *nad4* mtDNA gene following the protocol described in Martínez-Solano, Teixeira, Buckley and García-París (2006). Sequences were edited in SEQUENCHER (Gene Codes Corp., USA) and aligned by eye using MESQUITE 3.0.4 (Maddison & Maddison, 2015). Mitochondrial sequences were reduced to haplotypes using web-based tool FABOX 1.41 (http://users-birc.au.dk/biopv/php/fabox/) and the best-fit nucleotide substitution model (TN93+G) was selected based on the Bayesian information criterion (BIC) using JMODELTEST 2 (Guindon & Gascuel, 2003; Darriba, Taboada, Doallo & Posada, 2012). We carried out phylogenetic analyses based on Bayesian inference using BEAST 1.7 (Drummond, Suchard, Xie & Rambaut, 2012), specifying the optimal model of nucleotide substitution selected by JMODELTEST 2 and using the Bayesian skyline as the coalescent prior. Analyses were run for 30 million generations, sampling every 1000 generations. Convergence and stability of parameter estimates were assessed with TRACER 1.6 (Rambaut, Suchard, Xie & Drummond, 2006) and accordingly 15000 trees were discarded as burn-in.

We successfully sequenced and aligned 85 out of 87 samples with a length of 698 base pairs for *nad4* that were collapsed in 23 haplotypes. Phylogenetic analyses produced a gene tree with two main evolutionary lineages, comprising three and two sub-clades, respectively (Fig. A1.2). These results are in full agreement with major lineages as delineated by Martínez-Solano et al. (2006) using mitochondrial markers and by Teixeira et al. (2015) using the nuclear marker β-fibrinogen intron 7, indicating that our sampling was representative of the species phylogenetic diversity. Newly generated sequences have been deposited in GenBank (see Table A1.2 for accession numbers).

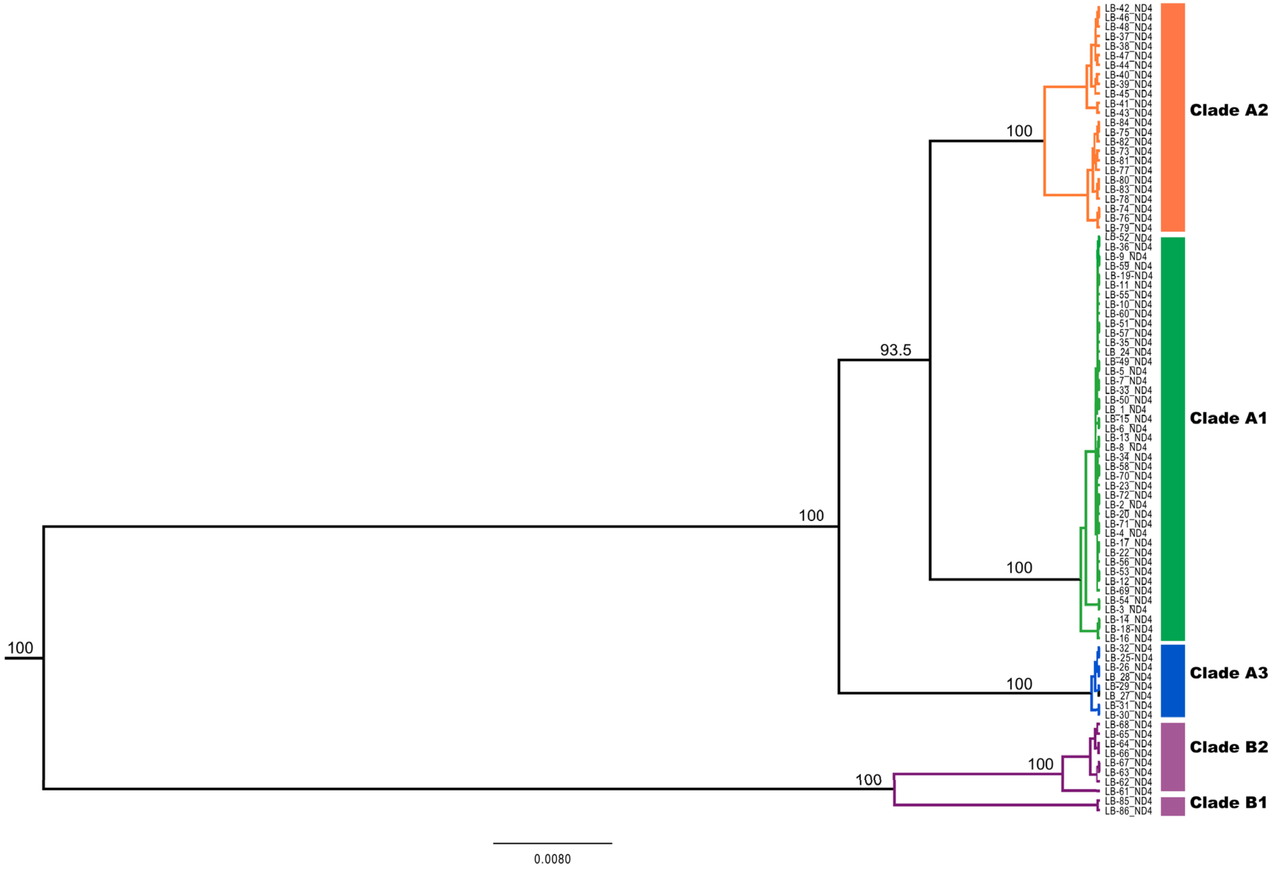

Figure A1.2 Bayesian gene tree of *Lissotriton boscai nad4* sequences recovered in populations studied. Colours represent the different lineages described in Martínez-Solano et al. (2006); we also follow their clade denominations. Numbers above the branches indicate Bayesian posterior probabilities (in %).

Table A1.1 Matrix of pairwise TN93+G mitochondrial genetic distance between sampled Iberian newt (*Lissotriton boscai*) populations.

	NAV	ESQ	VLC	ABL	NCD	PDC	SEC	MDC	MDS	TMB	PDP	ADR	CNT	VLD	RSV	GDO	BDR	MTC	SNT	NV	LDS	MCQ	FO
NAV	--																						
ESQ	0.002	--																					
VLC	0.002	0.003	--																				
ABL	0.009	0.010	0.009	--																			
NCD	0.010	0.010	0.010	0.001	--																		
PDC	0.009	0.010	0.009	0.000	0.001	--																	
SEC	0.030	0.031	0.031	0.023	0.024	0.023	--																
MDC	0.030	0.031	0.030	0.023	0.024	0.022	0.000	--															
MDS	0.030	0.031	0.030	0.023	0.024	0.022	0.000	0.000	--														
TMB	0.030	0.031	0.031	0.023	0.024	0.023	0.001	0.000	0.000	--													
PDP	0.030	0.031	0.030	0.023	0.024	0.022	0.000	0.000	0.000	0.000	--												
ADR	0.030	0.031	0.030	0.023	0.024	0.022	0.000	0.000	0.000	0.000	0.000	--											
CNT	0.030	0.031	0.030	0.023	0.024	0.022	0.000	0.000	0.000	0.000	0.000	0.000	--										
VLD	0.031	0.032	0.031	0.024	0.025	0.023	0.001	0.001	0.001	0.001	0.001	0.001	0.001	--									
RSV	0.030	0.031	0.030	0.023	0.024	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	--								
GDO	0.031	0.032	0.031	0.024	0.025	0.023	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	--							
BDR	0.030	0.031	0.031	0.023	0.024	0.023	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.001	0.000	0.001	--						
MTC	0.030	0.031	0.030	0.023	0.024	0.022	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001	0.000	--					
SNT	0.222	0.218	0.221	0.216	0.221	0.214	0.191	0.189	0.189	0.191	0.189	0.189	0.189	0.191	0.189	0.191	0.190	0.189	--				
NV	0.054	0.053	0.055	0.045	0.047	0.045	0.039	0.038	0.038	0.039	0.038	0.038	0.038	0.039	0.038	0.039	0.038	0.038	0.207	--			
LDS	0.056	0.053	0.057	0.046	0.047	0.046	0.039	0.039	0.039	0.039	0.039	0.039	0.039	0.040	0.039	0.040	0.039	0.039	0.211	0.001	--		
MCQ	0.209	0.202	0.208	0.202	0.203	0.201	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.175	0.173	0.175	0.174	0.173	0.033	0.182	0.182	--	
FO	0.211	0.205	0.210	0.204	0.206	0.203	0.173	0.173	0.173	0.173	0.173	0.173	0.173	0.175	0.173	0.175	0.174	0.173	0.034	0.183	0.184	0.003	--

Table A1.2 Population and individual codes for samples sequenced for the mitochondrial gene ND4, including the corresponding GenBank accession numbers.

Population	Population Name	Individual	GenBank Accession
1	Navia	LB-45	KY001974
1	Navia	LB-46	KY001975
1	Navia	LB-47	KY001976
1	Navia	LB-48	KY001977
2	Esqueiro	LB-41	KY001978
2	Esqueiro	LB-42	KY001979
2	Esqueiro	LB-43	KY001980
2	Esqueiro	LB-44	KY001981
3	Villacondide	LB-37	KY001982
3	Villacondide	LB-38	KY001983
3	Villacondide	LB-39	KY001984
3	Villacondide	LB-40	KY001985
4	Abelleira	LB-77	KY001986
4	Abelleira	LB-78	KY001987
4	Abelleira	LB-79	KY001988
4	Abelleira	LB-80	KY001989
5	Noceda	LB-73	KY001990
5	Noceda	LB-74	KY001991
5	Noceda	LB-75	KY001992
5	Noceda	LB-76	KY001993
6	Pobra do Caramiñal	LB-81	KY001994
6	Pobra do Caramiñal	LB-82	KY001995
6	Pobra do Caramiñal	LB-83	KY001996
6	Pobra do Caramiñal	LB-84	KY001997
7	Saelices el Chico	LB-57	KY001998
7	Saelices el Chico	LB-58	KY001999
7	Saelices el Chico	LB-59	KY002000
7	Saelices el Chico	LB-60	KY002001
8	Miranda del Castañar	LB-49	KY002002
8	Miranda del Castañar	LB-50	KY002003
8	Miranda del Castañar	LB-51	KY002004
8	Miranda del Castañar	LB-52	KY002005
9	Monforte de la Sierra	LB-53	KY002006
9	Monforte de la Sierra	LB-54	KY002007
9	Monforte de la Sierra	LB-55	KY002008
9	Monforte de la Sierra	LB-56	KY002009
10	El Tiemblo	LB-69	KY002010
10	El Tiemblo	LB-70	KY002011
10	El Tiemblo	LB-71	KY002012
10	El Tiemblo	LB-72	KY002013
11	Pelayos de la Presa	LB-01	KY002014
11	Pelayos de la Presa	LB-02	KY002015
11	Pelayos de la Presa	LB-03	KY002016
11	Pelayos de la Presa	LB-04	KY002017

Table A1.2 Continued

Population	Population Name	Individual	GenBank Accession
12	La Adrada	LB-05	KY002018
12	La Adrada	LB-06	KY002019
12	La Adrada	LB-07	KY002020
12	La Adrada	LB-08	KY002021
13	Cenicientos	LB-33	KY002022
13	Cenicientos	LB-34	KY002023
13	Cenicientos	LB-35	KY002024
13	Cenicientos	LB-36	KY002025
14	Valdastillas	LB-13	KY002026
14	Valdastillas	LB-14	KY002027
15	El Real de San Vicente	LB-23	KY002028
15	El Real de San Vicente	LB-24	KY002029
16	Garganta la Olla	LB-15	KY002030
16	Garganta la Olla	LB-16	KY002031
16	Garganta la Olla	LB-17	KY002032
16	Garganta la Olla	LB-18	KY002033
17	Los Baños de Robledillo	LB-09	KY002034
17	Los Baños de Robledillo	LB-10	KY002035
17	Los Baños de Robledillo	LB-11	KY002036
17	Los Baños de Robledillo	LB-12	KY002037
18	Montánchez	LB-19	KY002038
18	Montánchez	LB-20	KY002039
18	Montánchez	LB-22	KY002040
19	Sintra	LB-85	KY002041
19	Sintra	LB-86	KY002042
20	La Nava	LB-25	KY002043
20	La Nava	LB-26	KY002044
20	La Nava	LB-27	KY002045
20	La Nava	LB-28	KY002046
21	Linares de la Sierra	LB-29	KY002047
21	Linares de la Sierra	LB-30	KY002048
21	Linares de la Sierra	LB-31	KY002049
21	Linares de la Sierra	LB-32	KY002050
22	Monchique	LB-61	KY002051
22	Monchique	LB-62	KY002052
22	Monchique	LB-63	KY002053
22	Monchique	LB-64	KY002054
23	Foia	LB-65	KY002055
23	Foia	LB-66	KY002056
23	Foia	LB-67	KY002057
23	Foia	LB-68	KY002058

Appendix 1.3 Microsatellite markers amplification, genotyping, genetic diversity and population differentiation

Microsatellite markers amplification and genotyping

We used seven polymorphic microsatellite loci. Four loci were previously described in Sequeira, Silva-Ferreira and Lopes (2012) (Ltb4, Ltb10, Ltb11 and Ltb12), and three were specifically developed in this study: Ltb31 (Forward - 5’ - CAT TCA CAG GGT AAC AGA TGG – 3’; and Reverse 5’ - GCA TTT AAG GGT GGA TTG T – 3’); Ltb37 (Forward - 5’ - TGG AAT TAG AAG GCC AGT ACA – 3’, and Reverse 5’ - CTC TTC ACC CTT TAG GAG CA – 3’); and Ltb25 (Forward - 5’ – GCA TGA AGA CGA GGC TAA GA – 3’, and Reverse – 5’- GAT CTG GCT GGG ATA GAT AGA G – 3’). PCR amplifications were done following the conditions described in Sequeira et al. (2012). Microsatellite genotyping was accomplished with fluorescent-labelled primers. From PCR diluted product, we used 1 μL in combination with 10 μl of deionized formamide and 0.2 μl of internal size standard (Genescan-500 LIZ, ABI). Fragment size was determined on an ABI prism 3130XL capillary sequencer. Fragments were scored using GENEMAPPER 3.7 (Applied Biosystems) as described by Sequeira et al. (2012). Newly developed microsatellites sequences have been deposited in GenBank under accession numbers KY224074 (Ltb31), KY224075 (Ltb37) and KY224076 (Ltb25). Individual microsatellite genotypes are available from authors upon request.

Genetic diversity and population differentiation

Standard genetic diversity measures for polymorphic microsatellite markers were calculated using GENALEX 6.5 (Peakall & Smouse, 2006, 2012). We also performed tests for linkage disequilibrium (LD) and Hardy-Weinberg equilibrium (HWE) for each locus in the populations and global tests using the exact test of heterozygote deficiency and heterozygote excess implemented in GENEPOP 4.2 (Raymond & Rousset, 1995; Rousset, 2008) to detect deviations from HWE and avoid potential underestimations of genetic diversity (Table A1.3). All probability tests were based on MCMC simulations (Guo & Thompson, 1992; Raymond & Rousset, 1995) using default values, with significance levels adjusted for multiple tests using Bonferroni correction (Quinn & Keough, 2002).

Table A1.3 Populations of *Lissotriton boscai* sampled along the distributional range of the species including population code, latitude (Lat), longitude (Long), altitude (Alt), mitochondrial lineage and clade, mean snout-to-vent length (SVL), sampled size (n) and genetic diversity in the populations studied based on seven polymorphic microsatellite markers: average sample size over loci (*N*), number of alleles (*Na*), allelic richness (*Ar*), observed heterozygosity (*Ho*), and expected heterozygosity (*He*) in each population.

Population	Code	Latitude	Longitude	Altitude	Linage	Clade	Females		Males		*N*	*Na*	*Ar*	*Ho*	*He*
							SVL	n	SVL	n					
Navia	NAV	43.536	-6.719	29	A	A2	35.36	14	31.08	12	3.29	3.00	6.54	0.36	0.47
Esqueiro	ESQ	43.536	-6.268	139	A	A2	37.64	11	32.90	20	3.57	3.14	1.48	0.36	0.42
Villacondide	VLC	43.503	-6.740	75	A	A2	39.45	11	32.71	17	3.29	3.00	6.52	0.48	0.45
Abelleira	ABL	42.829	-9.035	164	A	A2	38.71	14	32.82	11	3.43	3.14	6.61	0.50	0.54
Noceda	NCD	42.696	-8.174	464	A	A2	43.25	4	34.33	3	3.43	3.71	6.65	0.61	0.57
Pobra do Caramiñal	PDC	42.632	-8.952	145	A	A2	37.50	10	31.82	22	3.43	3.00	6.56	0.54	0.49
Saelices el Chico	SEC	40.670	-6.627	659	A	A1	37.25	4	31.57	7	3.86	4.57	1.82	0.68	0.71
Miranda del Castañar	MDC	40.487	-5.993	584	A	A1	34.78	9	30.47	15	3.86	4.29	1.77	0.80	0.67
Monforte de la Sierra	MDS	40.485	-6.061	898	A	A1	36.26	19	31.80	10	3.71	4.43	1.76	0.61	0.66
El Tiemblo	TMB	40.389	-4.493	904	A	A1	36.33	3	33.17	6	3.43	3.00	1.55	0.56	0.48
Pelayos de la Presa	PDP	40.338	-4.359	803	A	A1	41.40	5	33.80	5	3.43	3.14	6.55	0.57	0.48
La Adrada	ADR	40.315	-4.643	672	A	A1	39.40	5	31.75	8	3.43	3.14	6.59	0.54	0.52
Cenicientos	CNT	40.256	-4.520	768	A	A1	36.13	16	33.00	8	3.43	3.14	6.59	0.54	0.51
Valdastillas	VLD	40.130	-5.867	726	A	A1	41.00	3	-	-	1.71	2.00	6.50	0.57	0.38
El Real de San Vicente	RSV	40.123	-4.709	706	A	A1	36.00	8	29.00	1	1.86	2.57	1.60	0.64	0.45
Garganta la Olla	GDO	40.108	-5.774	546	A	A1	35.38	8	30.78	9	3.57	3.00	1.52	0.46	0.46
Los Baños de Robledillo	BDR	39.503	-4.300	883	A	A1	42.10	10	33.60	5	4.00	3.71	1.68	0.50	0.60
Montánchez	MTC	39.227	-6.165	538	A	A1	36.50	10	31.50	8	3.71	3.57	1.68	0.50	0.59
Sintra	SNT	38.787	-9.429	302	B	B1	37.26	27	32.25	4	2.86	3.29	1.69	0.69	0.56
La Nava	NV	37.982	-6.749	373	A	A3	35.89	9	31.20	5	4.00	4.29	1.74	0.75	0.65
Linares de la Sierra	LDS	37.879	-6.647	658	A	A3	34.56	18	30.67	6	3.86	4.29	1.81	0.75	0.71
Monchique	MCQ	37.312	-8.529	716	B	B2	36.14	22	30.40	10	3.43	3.14	6.57	0.61	0.50
Foia	FO	37.309	-8.607	780	B	B2	36.80	15	32.10	10	3.29	3.00	6.63	0.57	0.55

Table A1.4 Matrix of pairwise F_{ST} values between sampled Iberian newt (*Lissotriton boscai*) populations. Values in bold denote F_{ST} values that are significantly different from zero.

	NAV	ESQ	VLC	ABL	NCD	PDC	SEC	MDC	MDS	TMB	PDP	ADR	CNT	VLD	RSV	GDO	BDR	MTC	SNT	NV	LDS	MCQ	FC
NAV	--																						
ESQ	0.014	--																					
VLC	0.023	0.018	--																				
ABL	**0.122**	**0.122**	**0.133**	--																			
NCD	0.052	0.051	0.038	-0.021	--																		
PDC	**0.145**	**0.146**	0.071	0.029	0.024	--																	
SEC	**0.099**	0.107	0.099	**0.093**	0.061	**0.126**	--																
MDC	**0.077**	**0.140**	**0.085**	**0.128**	**0.059**	**0.122**	0.064	--															
MDS	**0.067**	**0.101**	**0.056**	0.078	0.027	**0.107**	0.011	-0.025	--														
TMB	0.085	0.137	0.130	**0.139**	**0.074**	**0.171**	0.107	0.053	0.058	--													
PDP	**0.095**	**0.168**	**0.140**	**0.139**	0.039	**0.203**	**0.121**	**0.102**	**0.098**	0.088	--												
ADR	0.029	0.061	0.061	**0.110**	0.037	**0.111**	0.039	0.039	-0.009	-0.011	0.052	--											
CNT	0.059	0.073	0.050	**0.094**	0.018	**0.139**	**0.061**	0.017	-0.015	-0.022	0.043	-0.004	--										
VLD	0.194	0.242	0.247	0.160	0.066	0.221	0.127	0.130	0.074	0.102	0.185	0.047	0.131	--									
RSV	0.097	0.176	0.133	0.084	0.028	0.163	0.027	0.077	0.011	0.003	0.001	-0.049	0.005	0.033	--								
GDO	**0.113**	**0.144**	**0.110**	**0.161**	0.028	**0.201**	**0.154**	**0.102**	0.040	**0.126**	0.080	0.029	0.060	0.118	0.096	--							
BDR	**0.278**	**0.277**	**0.290**	**0.225**	**0.188**	**0.270**	**0.174**	**0.232**	**0.162**	**0.256**	**0.211**	**0.213**	**0.191**	0.274	0.187	**0.240**	--						
MTC	**0.249**	**0.284**	**0.332**	**0.195**	**0.213**	**0.295**	**0.159**	**0.205**	**0.186**	**0.217**	**0.241**	**0.188**	**0.248**	0.232	0.190	**0.278**	0.093	--					
SNT	**0.286**	**0.342**	**0.334**	**0.178**	**0.198**	**0.249**	0.127	**0.156**	0.128	**0.284**	**0.285**	**0.267**	**0.238**	0.360	0.304	**0.319**	**0.244**	**0.243**	--				
NV	**0.295**	**0.313**	**0.309**	**0.233**	**0.190**	**0.251**	**0.184**	**0.218**	**0.210**	**0.228**	**0.276**	**0.228**	**0.259**	0.251	0.218	**0.273**	**0.207**	**0.172**	**0.241**	--			
LDS	**0.258**	**0.287**	**0.280**	**0.221**	**0.168**	**0.250**	**0.146**	**0.180**	**0.167**	**0.252**	**0.258**	**0.216**	**0.220**	0.217	0.220	**0.241**	**0.174**	**0.153**	**0.214**	0.056	--		
MCQ	**0.291**	**0.279**	**0.309**	**0.267**	**0.295**	**0.329**	**0.226**	**0.269**	**0.264**	**0.369**	**0.366**	**0.338**	**0.321**	0.427	0.337	**0.349**	**0.270**	**0.287**	**0.302**	**0.312**	**0.250**	--	
FO	**0.213**	**0.226**	**0.231**	**0.194**	**0.220**	**0.242**	**0.143**	**0.194**	**0.188**	**0.310**	**0.320**	**0.273**	**0.258**	0.356	0.250	**0.315**	**0.225**	**0.253**	**0.228**	**0.266**	**0.195**	0.062	--

APPENDIX II

SUPPORTING INFORMATION

CHAPTER II

Assessing Rensch’s rule in a newt: roles of primary productivity and conspecific density in interpopulation variation of sexual size dimorphism

Miguel Peñalver-Alcázar, Pedro Galán & Pedro Aragón

Under review

Appendix 2.1 Field and literature-based population data

Table A2.1 Populations of *Lissotriton boscai* sampled during 2013 and 2015 along the distributional range of the species including population code (Code), latitude (Lat), longitude (Long), altitude (Alt), mitochondrial lineage and clade, mean snout-to-vent length (SVL), sampled size (n) and sexual dimorphism index (SDI) in each population.

Population	Code	Country	Lat	Lon	Alt	Lineage	Clade	Females				Males				SDI	
								2013		2015		2013		2015		2013	2015
								SVL	n	SVL	n	SVL	n	SVL	n		
Navia	NAV	Spain	43.536	-6.719	29	A	A2	35.36	14	36.14	36	31.08	12	31.83	18	0.14	0.14
Esqueiro	ESQ	Spain	43.536	-6.268	139	A	A2	37.64	11	38.67	33	32.90	20	33.33	43	0.14	0.16
Villacondide	VLC	Spain	43.503	-6.740	75	A	A2	39.45	11	41.20	5	32.71	17	34.56	9	0.21	0.19
Abelleira	ABL	Spain	42.829	-9.035	164	A	A2	38.71	14	39.54	13	32.82	11	33.91	11	0.18	0.17
Noceda	NCD	Spain	42.696	-8.174	464	A	A2	43.25	4	42.11	9	34.33	3	34.60	10	0.26	0.22
Pobra do Caramiñal	PDC	Spain	42.632	-8.952	145	A	A2	37.50	10	38.59	17	31.82	22	33.12	17	0.18	0.17
Saelices el Chico	SEC	Spain	40.670	-6.627	659	A	A1	37.25	4	-	-	31.57	7	-	-	0.18	-
Linares de Riofrío	LRF	Spain	40.582	-5.912	969	A	A1	-	-	34.50	2	-	-	30.60	5	-	0.13
La Honfría	HF	Spain	40.572	5.951	1100	A	A1	-	-	38.70	10	-	-	32.80	5	-	0.18
Miranda del Castañar I	MDCI	Spain	40.487	-5.993	584	A	A1	34.78	9	38.40	5	30.47	15	32.56	9	0.14	0.18
Miranda del Castañar II	MDCII	Spain	40.487	-5.994	586	A	A1	-	-	38.21	14	-	-	31.89	19	-	0.20
Monforte de la Sierra	MDS	Spain	40.485	-6.061	898	A	A1	36.26	19	38.07	28	31.80	10	31.90	48	0.14	0.19
El Tiemblo	TMB	Spain	40.389	-4.493	904	A	A1	36.33	3	35.00	1	33.17	6	33.25	4	0.10	0.05
Pelayos de la Presa	PDP	Spain	40.338	-4.359	803	A	A1	41.40	5	37.80	5	33.80	5	32.00	6	0.23	0.18
La Adrada	ADR	Spain	40.315	-4.643	672	A	A1	39.40	5	-	-	31.75	8	-	-	0.24	-
Cenicientos	CNT	Spain	40.256	-4.520	768	A	A1	36.13	16	37.25	4	33.00	8	33.33	9	0.10	0.12
El Real de San Vicente	RSVI	Spain	40.123	-4.709	706	A	A1	36.00	8	37.67	3	29.00	1	31.00	3	0.24	0.22
El Real de San Vicente	RSVII	Spain	40.123	-4.709	706	A	A1	-	-	38.63	8	-	-	30.88	16	-	0.25
Garganta la Olla	GDO	Spain	40.108	-5.774	546	A	A1	35.38	8	35.77	48	30.78	9	32.06	51	0.15	0.12

Table A2.1 Continued

Population	Code	Country	Lat	Lon	Alt	Lineage	Clade	Females				Males				SDI	
								2013		2015		2013		2015		2013	2015
								SVL	n	SVL	n	SVL	n	SVL	n		
Los Baños de Robledillo	BDR	Spain	39.503	-4.300	883	A	A1	42.10	10	35.50	2	33.60	5	34.75	4	0.25	0.02
Montánchez	MTC	Spain	39.227	-6.165	538	A	A1	36.50	10	37.62	13	31.50	8	31.60	10	0.16	0.19
Sintra	SNT	Portugal	38.787	-9.429	302	B	B1	37.26	27	-	-	32.25	4	-	-	0.16	-
Aldeaquemada	ALQ	Spain	38.398	-3.403	787	A	A1	-	-	37.78	9	-	-	33.08	12	-	0.14
La Nava	NV	Spain	37.982	-6.749	373	A	A3	35.89	9	36.50	4	31.20	5	32.50	2	0.15	0.12
Linares de la Sierra	LDS	Spain	37.879	-6.647	658	A	A3	34.56	18	36.67	3	30.67	6	32.00	3	0.13	0.15
Monchique	MCQ	Portugal	37.312	-8.529	716	B	B2	36.14	22	-	-	30.40	10	-	-	0.19	-
Foia	FOIA	Portugal	37.309	-8.607	780	B	B2	36.80	15	-	-	32.10	10	-	-	0.15	-

Table A2.2 Populations of *Lissotriton boscai* gathered through literature review of previous studies including population name, country, year, mean snout-to-vent length (SVL), sampled size (n) and the original source of the information.

Population	Country	Year	Females		Males		Reference
			SVL	n	SVL	n	
Miranda del Castañar	Spain	2009	39.03	87	34.95	87	Ortiz-Santaliestra, Marco, Fernández-Benéitez and Lizana (2009)
Doñana	Spain	1999	27.20	16	25.10	22	Díaz-Paniagua and Mateo (1999)
Huelva-Aroche	Spain	1999	38.28	37	33.19	25	Díaz-Paniagua and Mateo (1999)
Pontevedra-Bueu	Spain	1999	43.12	37	36.84	26	Díaz-Paniagua and Mateo (1999)
Lugo-Ancares	Spain	1999	41.57	33	35.63	29	Díaz-Paniagua and Mateo (1999)
Salamanca-Candelario	Spain	1999	37.97	37	32.58	33	Díaz-Paniagua and Mateo (1999)
Ons	Spain	2007	40.35	45	36.23	63	Rivera, Velo-Antón and Galán (2007)
Sálvora	Spain	2007	40.62	2	35.69	5	Rivera *et al.* (2007)
Cabo Udra	Spain	2007	44.86	4	38.37	12	Rivera *et al.* (2007)
Castro Labor	Spain	1982	38.57	21	32.25	12	Caetano (1982)
Tourém	Portugal	1982	35.74	29	30.89	18	Caetano (1982)
Mezio	Portugal	1982	36.01	64	31.57	85	Caetano (1982)
San Joao Campo	Portugal	1982	35.59	15	31.68	38	Caetano (1982)
Malhadoura	Portugal	1982	37.30	10	32.26	21	Caetano (1982)
Fafiao	Portugal	1982	37.64	14	32.60	10	Caetano (1982)

Appendix 2.2 Analyses excluding populations with low samples size

As several field-sampled populations had low sample size (number of females or males per population was 4 or even 2), statistical analyses were performed including only those populations with at least 5 individuals of each sex, i.e., the smallest included population had 5 males and 5 females. Therefore, the total number of populations (N) used for Model I and Model II regressions as well as PLSR analysis for populations sampled in 2013 was N = 17, total N for Model I and Model II regressions for the combined field and literature populations was N = 35, and total N for Linear mixed models and model selection was N = 15.

Model I and Model II regressions

Model I regression showed a significant relationship between the mean sizes of the sexes across populations, and this relationship differed significantly from 1 for populations sampled in 2013 ($R^2 = 0.67$, slope = 0.47 [95% *CI* = 0.29-0.66], $P < 0.001$, Fig. A2.1a) and in the combined field and literature populations ($R^2 = 0.83$, slope = 0.75 [95% *CI* = 0.63-0.88], $P < 0.001$, Fig. A2.1b). Model II regression revealed similar results for populations sampled in 2013 ($R^2 = 0.67$, slope = 0.52 [95% *CI* = 0.33-0.74], $P < 0.001$, Fig. A2.1a) and the combined field and literature populations ($R^2 = 0.83$, slope = 0.81 [95% CI = 0.68-0.96], P = 0.014, Fig. A2.1b).

These results are in line with those yielded by the Model I and Model II regressions performed including all populations.

PLSR analysis

PLSR analysis on the pruning 2013 dataset provided three significant components explaining 91.9% of the original variance in SDI for the 17 populations (PLRS-1: $r = 0.89$, $P < 0.0001$; PLRS-2: $r = 0.62$, $P = 0.008$; PLRS-3: $r = 0.58$, $P = 0.014$; Table A2.3). Relative female density, EVI seasonality, mean EVI and capture season were the most relevant ecological predictors for explaining SDI according to VIP (> 1 in 4 out of 10 cases, Table A2.3) and information explained in the PLSR-1 (> 10% in 3 out of 10 cases, Table A2.3) and PLSR-2 (> 10% in 2 out of 4 cases, Table A2.3).

Relative males density, annual mean temperature, altitude, temperature seasonality and annual precipitation showed a moderate influence, since their VIP was < 1 and explained information was only > 10% in PLSR-2 and PLSR-3, which together accounted for 11.3% out of 91.9% of the original variance in SDI (Table A2.3). Precipitation seasonality was not considered relevant according to VIP (< 1 and information explained in PLSR (< 10%). Only F_{ST}-PV12 (phylo)genetic predictor was also relevant to explain SDI, yielding VIP > 1 and explained information > 10% in the PLSR-1 component (Table A2.3).

Relative female density was a highly relevant predictor of SDI (VIP = 2.0, Table A2.3) and showed the strongest negative relationship with SDI in PLSR-1 (explained information in PLSR-1 = 27%, Table A2.3, Fig. A2.2a). Relative male density showed a moderate influence (VIP = 0.9 and information explained in PLSR > 10% in PLSR-2, Table A2.3, Fig. A2.2b). Mean EVI showed a positive relationship with the response variable in PLSR-1 and PLSR-2, whereas EVI seasonality was negatively and positively related with the response variable in PLSR-1 and PLSR-2, respectively (Fig. A2.2a,b). Both primary productivity-related variables were amongst the most relevant predictors for SDI (Table A2.3). The contribution of capture season was also noticeable (VIP = 1.2, explained information in PLSR-1 = 10.2% and PLSR-2 = 11.5%, Fig. A2.2a,b).

Linear mixed models and model selection

Model selection in linear mixed model for the SDI on the 2013+2015 dataset excluding population with extremely low samples size provided only one model for model averaging according to the cut-off criterion of ΔAICc < 2. According to this model, SDI was best explained by Mean EVI, relative female density and altitude.. Relative female density was negatively related to SDI (estimate ± SE = 0.022037 ± 0.004627, 95% CI = -0.031 – -0.013), whereas EVI mean and altitude were positively related to SDI (estimate ± SE = 0.021689 ± 0.006212, 95% CI = 0.009-0.034; estimate ± SE = 0.020589 ± 0.006190, 95% CI = 0.008-0.033, respectively).

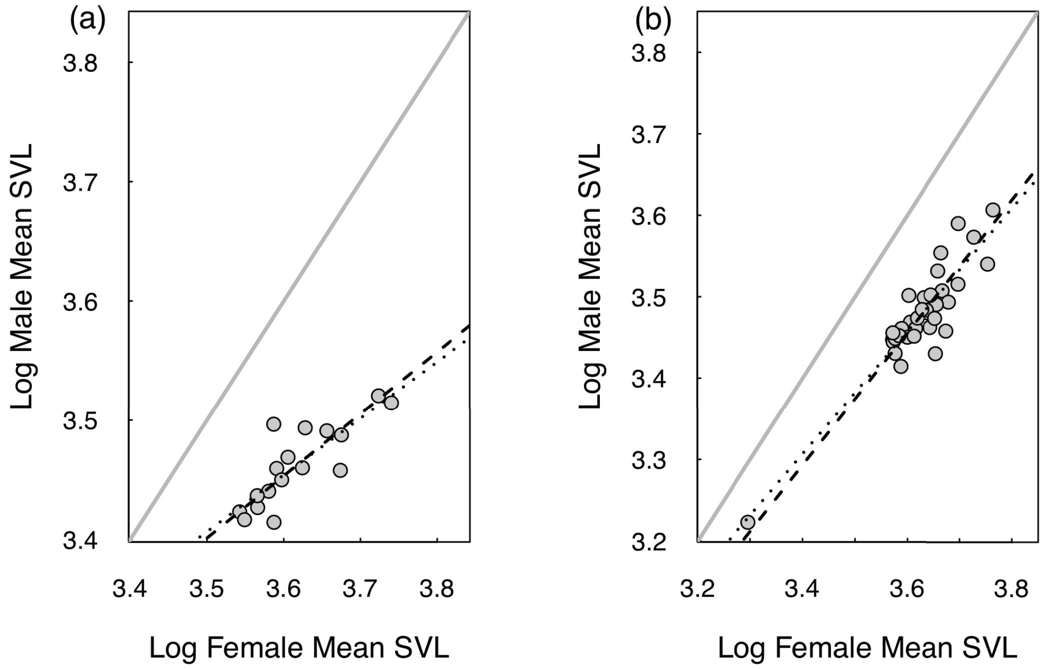

Figure A2.1 Log male mean snout-vent length (SVL) versus log female mean SVL for (**a**) 17 field populations of *Lissotriton boscai* sampled in 2013 with Model I regression dotted line (slope = 0.47 [95% CI = 0.29-0.66]) and Model II regression dashed line (slope = 0.52 [95% CI = 0.33-0.74]) and (**b**) 42 field (2013 and 2015) and literature populations of *L. boscai* with Model I regression dotted line (slope = 0.75 [95% CI = 0.63-0.88]) and Model II regression dashed line (slope = 0.81 [95% CI = 0.68-0.96]). The thick grey line represents isometry, i.e., slope = 1. Each dot represents a single population.

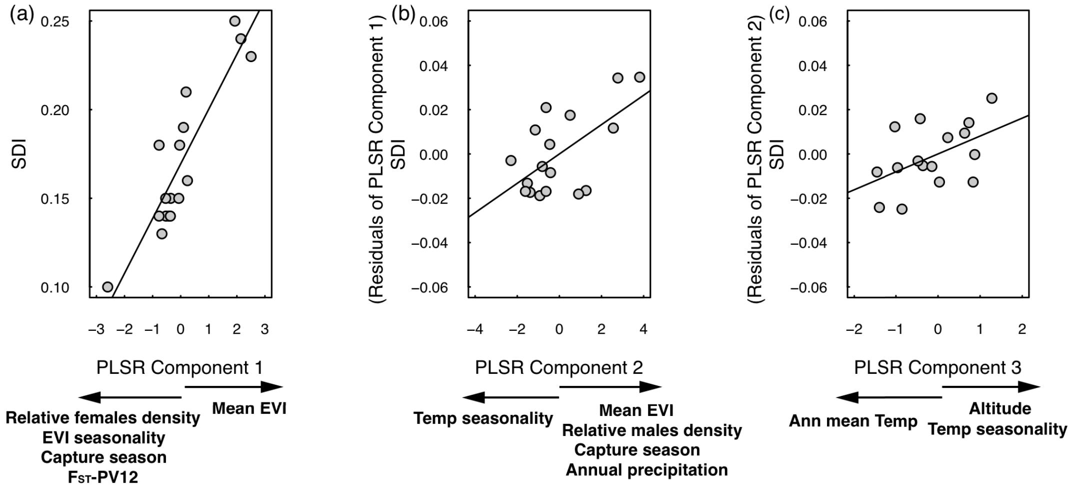

Figure A2.2 Relationships between sexual dimorphism index (SDI) for 17 field populations of the Iberian newt sampled in 2013 and the first (a), second (b) and third (c) relevant partial least squares regression (PLSR) components. Predictors with explained information > 10% in each PLSR component and variable importance for projection (VIP) > 1 are shown in the x-axes (see Table A2.2). Each dot represents a single population.

Table A2.3 Predictor weights for selected components of partial least squares regression (wPLSR) of sexual dimorphism index (SDI) for 17 field populations of the Iberian newt sampled in 2013 and their variable importance for projection (VIP). R^2 is the proportion of the original variance of SDI explained by each PLSR component. Bold lettering denotes predictors with explained information (square of predictor weights*100) > 10% or VIP > 1.

Predictor variable	VIP	wPLSR-1	wPLSR-2	wPLSR-3
Ecological predictors				
Relative females density	**2.0**	**-0.52**	0.12	0.19
EVI seasonality	**1.6**	**-0.43**	-0.28	-0.25
Capture season	**1.2**	**-0.32**	**0.34**	0.02
Mean EVI	**1.2**	0.24	**0.54**	-0.01
Relative males density	0.9	-0.20	**0.45**	0.23
Annual mean temperature	0.8	-0.16	-0.19	**-0.39**
Precipitation seasonality	0.6	-0.15	-0.10	0.13
Altitude	0.6	0.10	-0.14	**0.58**
Temperature seasonality	0.5	0.06	**-0.31**	**0.39**
Annual precipitation	0.4	-0.07	**0.34**	-0.28
(Phylo)genetic predictors				
FST-PV12	**1.7**	**-0.47**	0.03	0.28
FST-PV11	0.7	0.16	0.12	0.17
nad4-PV8	0.7	0.18	0.08	0.04
R^2 by the PLSR component		0.801	0.073	0.041

Appendix 2.3 Analyses using averages of body size (SVL) and sexual dimorphism index (SDI)

We first run an OLS and MA analyses using the female and male average SVL across years for those populations sampled in both years (i.e., 2013 and 2015) with enough sample size. Model I and Model II regressions showed a significant relationship between the mean sizes of the sexes across populations, and this relationship differed significantly from 1 (Model I regression results: $R^2 = 0.75$, slope = 0.54 [95% CI = 0.35-0.73], $P < 0.001$, Fig. A2.3; Model II regression results: $R^2 = 0.75$, slope = 0.59 [95% CI = 0.39-0.82], $P = 0.004$; Fig. A2.3). These results are in line with those yielded by the analyses either excluding or including populations with low sample size.

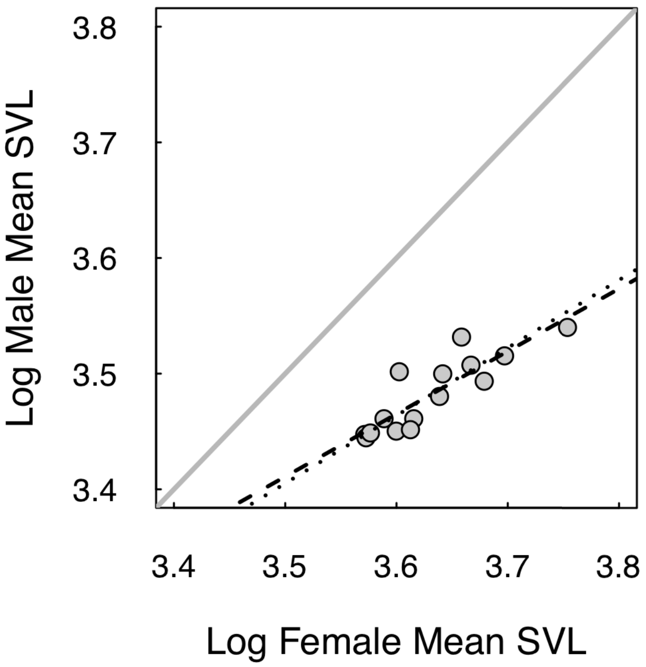

Figure A2.3 Log male mean snout-vent length (SVL) versus log female mean SVL for 15 field populations of *Lissotriton boscai* sampled both in 2013 and 2015 with Model I regression dotted line (slope = 0.54 [95% CI = 0.35-0.73], $P < 0.001$) and Model II regression dashed line (slope = 0.59 [95% CI = 0.39-0.82], $P = 0.004$). The thick grey line represents isometry, i.e., slope = 1. Each dot represents a single population.

Then, we fit a linear mixed model with the average SDI across years only for those populations sampled in both years (i.e., 2013 and 2015) with enough sample size. Average SDI was used as dependent variable. Altitude, capture season, relative females and males density, EVI mean, EVI seasonality, annual mean temperature, annual precipitation, temperature seasonality and precipitation seasonality were included as fixed factor variables and population as random factor. The procedure used was similar to the one described in the main text. In brief, all possible models were fitted using maximum likelihood, keeping the same random effects structure for all models (i.e., population). The second-order AIC corrected for small sample size (AICc) and AIC weight (AICw) scores were calculated for all possible models. The cut-off criterion of $\Delta AICc < 2$ was used to delineate a "top model set". Model-averaged parameter estimates, standard errors, confidence intervals and relative variable importance were computed for the fixed factors in all selected models. Estimates and standard errors of weighed parameters were calculated following the "zero average method". Predictors were standardized to avoid scale and unit differences. Model selection in linear mixed model for the average SDI across years yielded 3 models for model averaging according to the cut-off criterion of $\Delta AICc < 2$. The most relevant parameters for explaining SDI were Mean EVI and relative female density. EVI mean was present in 2 out of 3 models (relative importance = 0.78) and relative female density was present in 1 out of 3 models (relative importance = 0.45). Annual precipitation was also present in 1 out of 3 models (relative importance = 0.22). Relative female density was negatively related to SDI (estimate ± SE = -0.007 ± 0.008, 95% CI = -0.024183769–0.01085785), whereas EVI mean was positively related to SDI (estimate ± SE = 0.014 ± 0.009, 95% CI = -0.005594289–0.03331682). Annual precipitation was also positively related to SDI (estimate ± SE = 0.017340 ± 0.008, 95% CI = -0.012230614–0.0197260). Overall, these results are in line with those presented in the main text and support the conclusions drawn from them.

Appendix 2.4 Courtship behaviour experiment in *Lissotriton boscai* (Aragón, 2009)

The courtship behaviour experiment was originally performed to investigate the male newts behavioural displays in response to different social environments. For this, 21 males and 20 females were collected in a natural population in Spain (Navia, Asturias) in February 2008. Newts were measured for snout-to-vent length (SVL) to the nearest 0.5 mm (males: mean ± SE = 69 ± 0.86 mm, females: mean ± SE = 75.85 ± 0.78 mm) and individually housed in water filled aquaria for two weeks. Male-female encounters (one-to-one) were staged and the behavioural responses of males and females were recorded (see Aragón, 2009 for full details). For the current study, we revisited the female avoidance behaviour (i.e., number times that a female withdrew or slowly moved away from the male) and activity (i.e., total time spent moving by a female) and retrieved previously not performed analyses of the female avoidance events as a function of male SVL and total length for the twenty male-female pairs used in the experiment (Table A2.4). Then, we assessed whether female avoidance behaviour during male courtships was related to male morphological traits by means of Pearson correlation tests. We found no significant correlation between female avoidance events and male SVL (Pearson correlation: $F_{1,18} = 0.62$, $r = 0.18$, $P = 0.44$; Fig. A2.4a) or total length ($F_{1,18} = 0.42$, $r = -0.15$, $P = 0.52$; Fig. A2.4b).

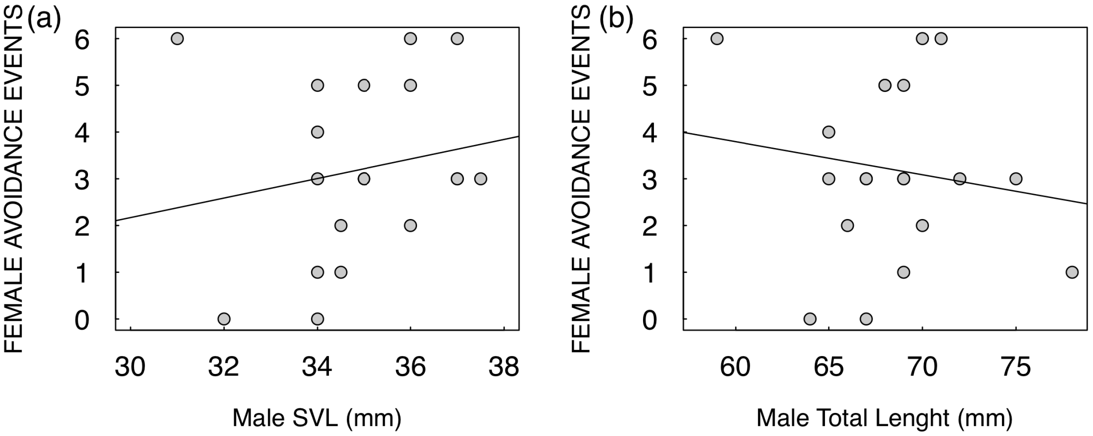

Figure A2.4 Relationship between number of female avoidance events and (**a**) male snout-to-length (SVL) and (**b**) total length. None of these correlations were significant.

Table A2.4 Data on female behavioural responses and male mean snout-to-vent length (SVL) and total length from the courtship experiment described in Aragón (2009).

Male ID	Female ID	Female avoidance events	Male SVL	Male total length
12	4	0	32	64
13	9	5	34	68
9	8	0	34	67
10	1	6	36	70
7	5	3	34	65
2	12	3	35	67
8	10	5	35	68
3	11	1	34	78
5	6	2	36	70
1	2	3	37.5	72
6	16	3	37	75
16	17	6	37	71
4	20	3	37	69
17	18	5	36	69
14	19	2	34.5	66
20	14	4	34	65
11	3	3	35	67
19	13	1	34.5	69
21	15	3	35	69
15	7	6	31	59

Appendix 2.5 Reproductive investment in *Lissotriton boscai* (Brea, Galán, Ferreiro & Serantes, 2007)

Fecundity data of *Lissotriton boscai* was collected during three entire reproductive cycles (November 2002 to August 2005) and some additional data gathered during the reproductive cycle of 2009 (unpublished), under controlled and natural conditions. Females were sampled at the beginning of each reproductive cycle from a natural population in Parque Natural de las Fragas do Eume (Galicia, Spain). The population was monthly monitored using a capture-mark recapture method. This procedure enabled us to precisely detect females that had just arrived at the aquatic habitat to start oviposition. Females were kept under natural light and temperature conditions in plastic containers of 40-liter capacity set up with an artificial oviposition support consisting in several plastic strips (35x2 cm). Aquarias were daily checked and eggs number and diameter recorded. Females were measured for snout-vent-length (SVL) with an accuracy of 0.1 mm. and the eggs were measured without the capsule with an accuracy of 0.01 mm. Furthermore, the number of oocytes undergoing vitellogenesis process in the ovaries of dissected females was counted. We decided to count the number of oocytes because previous studies using newts as a models species found a correlation between female body size and the number of oocytes (Verrell, Halliday & Griffiths, 1986; Nobili & Accordi, 1997). Females were captured at the very moment of their arrival to the breeding aquatic habitat (November to February in 2000-2001). The time period selected for the dissection of the females and the oocyte count was the beginning of the laying period in the study area (Lizana, Ciudad & Pérez-Mellado, 1989; Galán, 2017). The dissected newts were deposited in the laboratory of Zoology II of the Department of Biology, in the Faculty of Sciences of the University of A Coruña (Campus A Zapateira, s / n, 15071-A Coruña, Spain).

Overall, the reproductive investment data collected included: the total number of ovoposited eggs for the 11 females that were considered to have completed their total laying of the season (> 30 days in the aquatic habitat), the mean number of ovoposited eggs per day for all the 23 females, the average size of eggs for 18 females, and the total number of oocytes for 27 females (Table A2.5). For more details, see Brea et al. (2007).

For the current study, we revisited the female fecundity and SVL data described above and assessed the previously not explored relationship between female body size and different parameters of fecundity, namely, total number of ovoposited eggs (i.e., clutch size), number of ovoposited eggs per day, diameter of eggs, and total number of oocytes by means of Pearson correlation tests. We found no significant correlation between female SVL and number of eggs ($F_{1,9} = 0.03$, $r = -0.06$, $P = 0.86$; Fig. A2.5a), number of eggs per day ($F_{1,21} = 0.62$, $r = 0.17$, $P = 0.44$; Fig. A2.5b), diameter of eggs ($F_{1,16} = 2.89$, $r = 0.39$, $P = 0.11$; Fig. A2.5c) and number of oocytes ($F_{1,25} = 2.27$, $r = 0.29$, $P = 0.14$; Fig. A2.5d). Finally, it is noteworthy to indicate that in this species the number of oocytes was significantly higher than the clutch size (Brea et al., 2007).

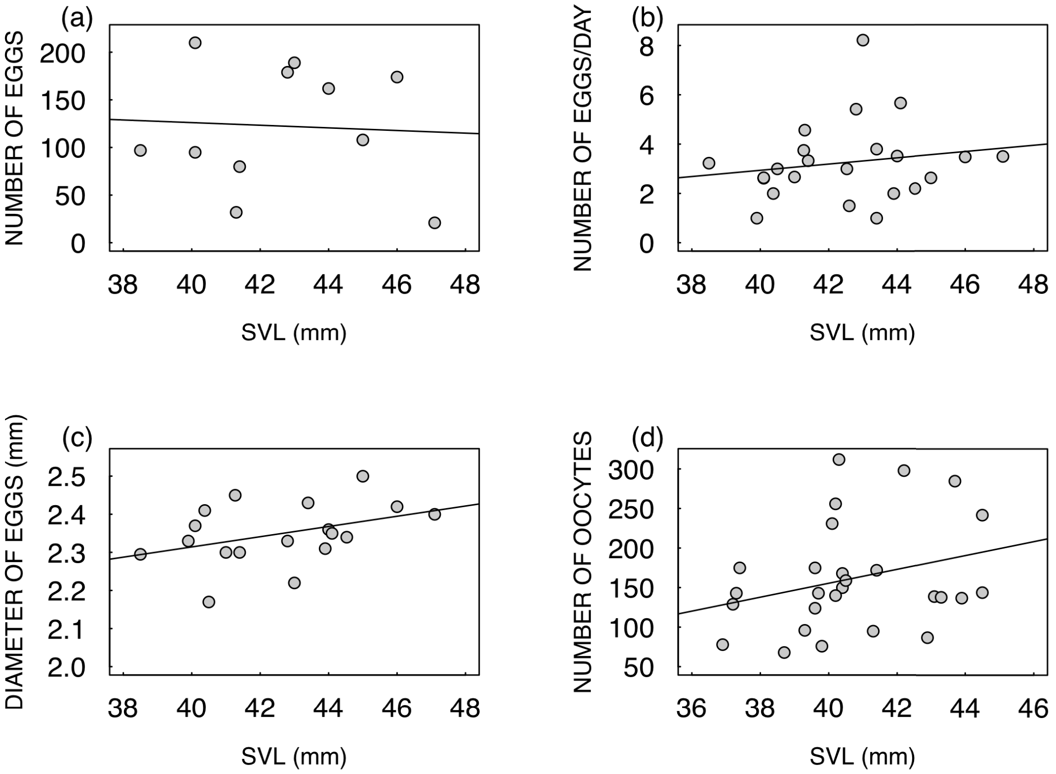

Figure A2.5 Relationship between female snout-vent-length (SVL) and (**a**) number of eggs, (**b**) number of eggs per day, (**c**) diameter of eggs and (**d**) number of oocytes. None of these correlations were significant.

Table A2.5 Data on female fecundity parameters and snout-to-vent length (SVL) from the experiment described in Brea et al (2007) with additional data from 2009.

Female ID	Female SVL	Number of eggs	Number of eggs/day	Mean egg diameter	Egg laying period	Female ID	Female SVL	Number of oocytes	Month
1	41.4	80	3.33	2.3	Feb. - Apr. 2003	1	40.4	168	November 2000
2	42.8	179	5.42	2.33	Feb. - May 2005	2	41.3	95	November 2000
3	43	189	8.22	2.22	Feb. - Apr. 2005	3	36.9	78	November 2000
4	46	174	3.48	2.42	Feb. - May 2003	4	43.9	137	November 2000
5	45	108	2.63	2.5	Feb. - Apr. 2003	5	38.7	68	November 2000
6	40.1	210	2.62	2.37	Dec. 2002 - Apr. 2003	6	42.9	87	November 2000
7	38.5	97	3.23	2.295	Feb. - Apr. 2009	7	37.4	175	November 2000
8	44	162	3.52	2.36	Feb. - Apr. 2009	8	40.4	150	November 2000
9	41.3	32	4.57	-	Feb. - Mar. 2005	9	37.2	129	November 2000
10	47.1	21	3.5	2.4	Feb. - Mar. 2003	10	39.7	143	December 2000
11	40.1	95	2.64	-	Feb. - May 2004	11	39.8	76	December 2000
12	41.27	-	3.75	2.45	Feb. - Mar. 2005	12	40.3	312	December 2000
13	43.4	-	1	2.43	Feb. - Mar. 2004	13	43.1	139	December 2000
14	43.9	-	2	2.31	Feb. 2003	14	40.5	159	December 2000
15	40.38	-	2	2.41	Feb. - Mar. 2004	15	39.6	175	December 2000
16	39.9	-	1	2.33	Mar. 2004	16	40.1	231	December 2000
17	41	-	2.67	2.3	Mar. 2004	17	40.2	140	December 2000
18	43.4	-	3.8	2.34	Mar. 2004	18	43.7	285	January 2001
19	42.6	-	1.5	-	Mar. 2004	19	37.3	143	January 2001
20	44.53	-	2.2	-	Feb. 2005	20	44.5	144	January 2001
21	42.53	-	3	-	Feb. 2005	21	39.3	96	January 2001
22	44.1	-	5.67	2.35	Feb. - Apr. 2004	22	41.4	172	January 2001
23	40.5	-	3	2.17	Feb. - Mar. 2005	23	42.2	298	January 2001
						24	43.3	138	February 2001
						25	40.2	256	February 2001
						26	44.5	242	February 2001
						27	39.6	124	February 2001

Appendix 2.6 Spatial autocorrelation in PLSR analysis

Ecological variables commonly exhibit spatial autocorrelation, which it is defined as the non-independence between two groups of observations with a certain distance in space (Legendre, 1993; Borcard et al., 2004). The results of statistical models can be affected by the presence of spatial autocorrelation in the data, resulting in biased and/or reversed parameter estimates and/or overestimations of the environmental factors contribution (Legendre et al., 2002). Residuals from the PLSR models were explored for the presence of spatial autocorrelation using the Moran's *I* coefficient (199 permutations and sequential Bonferroni correction used to test significance) and spatial correlograms (see Fig. A2.6 and Fig. A2.7) in SAM (Rangel et al., 2010).

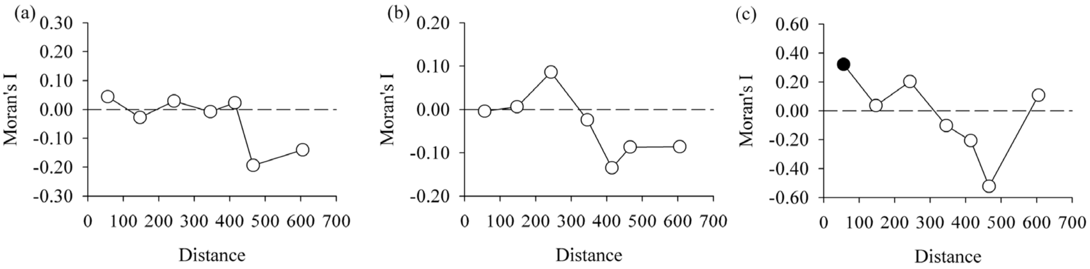

Figure A2.6 Spatial correlograms on partial least squares regression (PLSR) model residuals for sexual dimorphism index first (**a**), second (**b**) and third (**c**) relevant PLSR components. Open circles indicate no-significant correlation and solid black circles indicate negative correlations after sequential Bonferroni corrections.

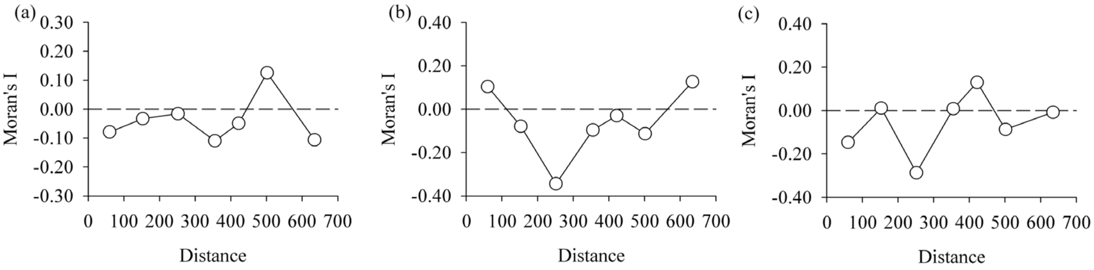

Figure A2.7 Spatial correlograms on partial least squares regression (PLSR) model residuals for sexual dimorphism index first (**a**), second (**b**) and third (**c**) relevant PLSR components for 17 out of 22 populations sampled in 2013. Open circles indicate no-significant correlation after sequential Bonferroni corrections.

Appendix 2.7

Table A2.6 Model comparison for effects of ecological parameters on Sexual Dimorphism Index (SDI) for 2013 and 2015 *Lissotriton boscai's* populations combined data. Displayed are the full model set of the 13 submodels selected using the cut-off criterion of ΔAICc < 2, including model number (Model ID), intercept, altitude (Alt), annual mean temperature (Anmt), annual mean precipitation (Anmpr), capture season, mean primary productivity (Mean EVI), primary productivity seasonality (EVI seaso), females relative density (Femrd), precipitation seasonality (Prseaso), degrees of freedom (df), log likelihood (logLik), second-order AIC corrected for small sample size (AICc) and AIC weight (AICw).

Model ID	Intercept	Alt	Anmt	Anmpr	Capture season	MeanEVI	EVIseaso	Femrd	Prseaso	df	logLik	AICc	ΔAICc	AICw
121	0.1661				-0.0278	0.0279	0.0138	-0.0159		8	81.292	-142.5	0	0.137
283	0.1661		-0.0200		-0.0331	0.0308			0.0137	8	80.864	-141.6	0.86	0.089
122	0.1661	0.0121			-0.0290	0.0359	0.0133	-0.0136		9	82.452	-141.6	0.86	0.089
26	0.1663	0.0157			-0.0247	0.0341				7	79.336	-141.6	0.91	0.087
89	0.1661				-0.0236	0.0243		-0.0114		7	79.203	-141.3	1.17	0.076
27	0.1665		-0.0123		-0.0276	0.0262				7	79.175	-141.2	1.23	0.074
126	0.1661	0.0187		0.0161	-0.0336	0.0302	0.0141	-0.0132		10	83.911	-141.2	1.31	0.071
123	0.1661		-0.0089		-0.0307	0.0295	0.0121	-0.0145		9	82.221	-141.1	1.32	0.071
91	0.1661		-0.0110		-0.0279	0.0268		-0.0103		8	80.569	-141	1.44	0.066
25	0.1667				-0.0229	0.0232				6	77.626	-141	1.49	0.065
30	0.1661	0.0219		0.0155	-0.0289	0.0283				8	80.506	-140.9	1.57	0.062
379	0.1661		-0.0160		-0.0357	0.0336	0.0127	-0.0125	0.0122	10	83.724	-140.8	1.69	0.059
90	0.1661	0.0129			-0.0251	0.0329		-0.0092		8	80.396	-140.7	1.79	0.056

Random terms: 1| Pop + 1| Year

APPENDIX III

SUPPORTING INFORMATION

CHAPTER III

Niche differentiation between deeply divergent phylogenetic lineages of an endemic newt: implications for Species Distribution Models

Miguel Peñalver-Alcázar, Alberto Jiménez-Valverde & Pedro Aragón
Under review

Appendix 3.1 Predictive maps derived from Species Distribution Models

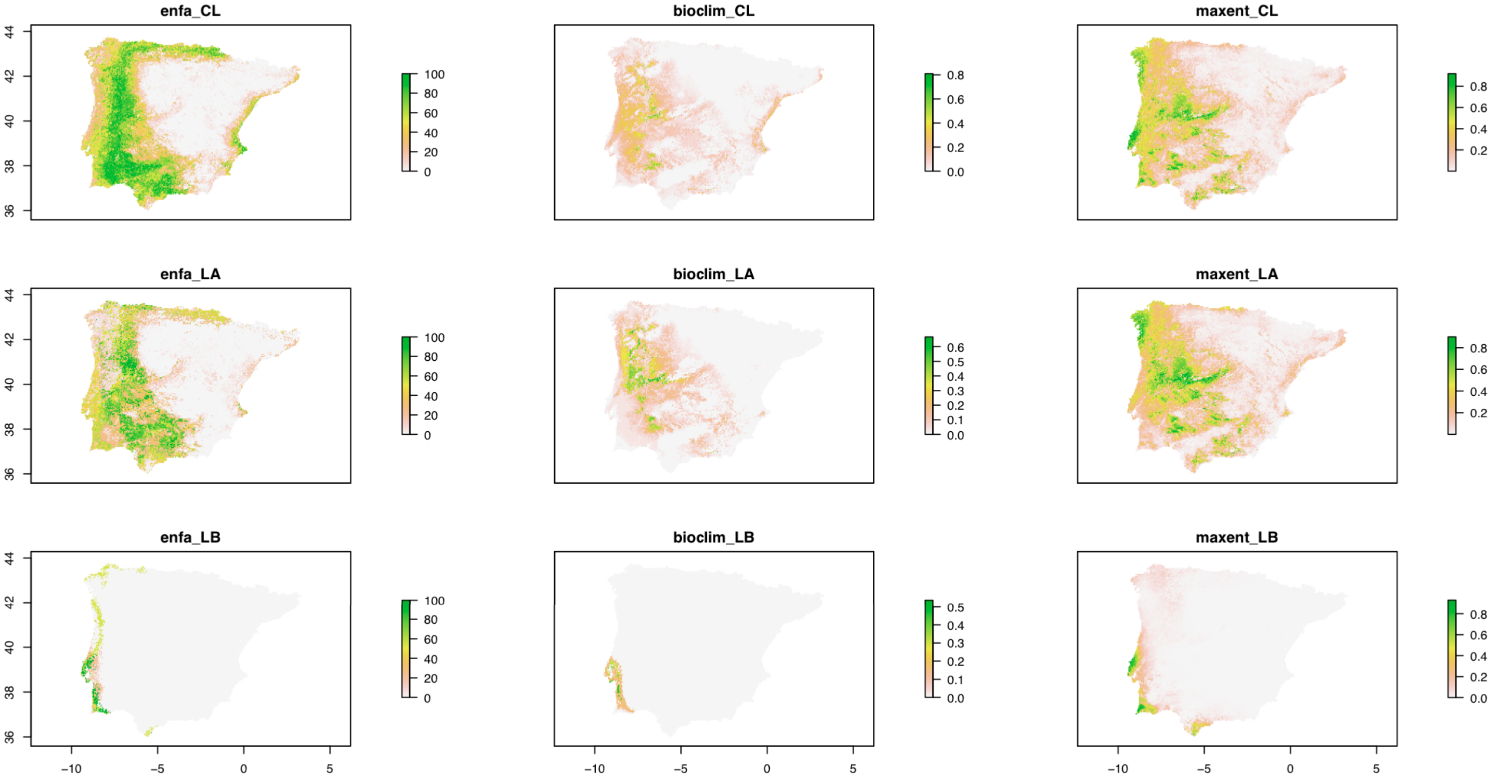

Figure A3.1 Predictive maps derived from ENFA (left), Bioclim (centre) and MaxEnt (right) models for combined lineages (SP), lineage A (LA) and lineage B (LB).

Appendix 3.2 Principal Component Analysis (PCA) on the environmental variables related to the distribution of *Lissotriton boscai* phylogenetic lineages

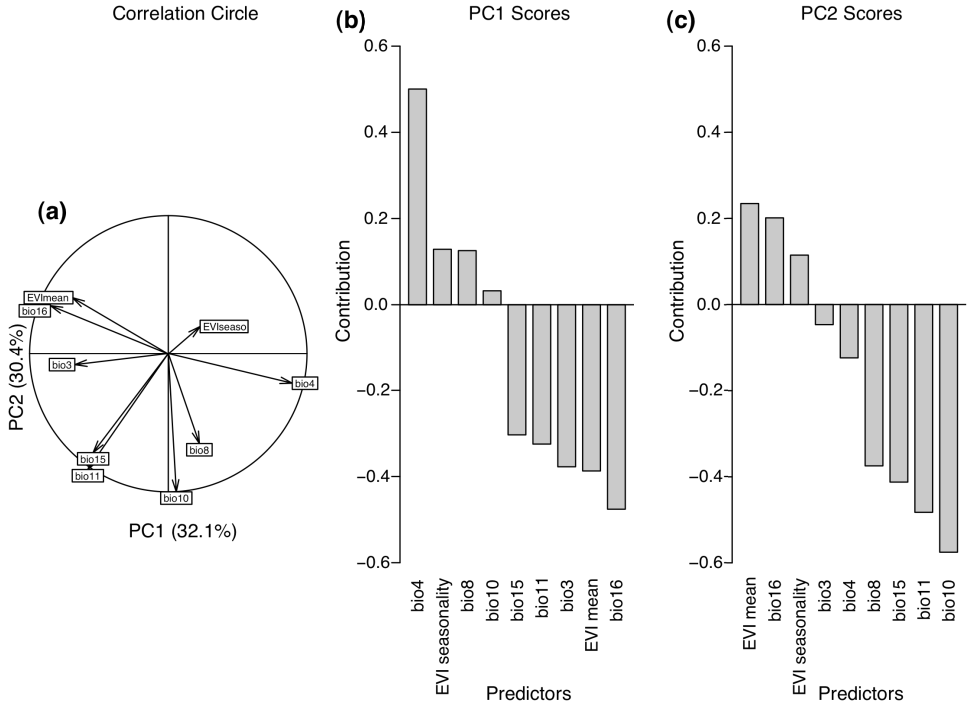

Figure A3.2 Results of the Principal Component Analysis (PCA) on the environmental variables related to the distribution of *Lissotriton boscai* phylogenetic lineages: (**a**) variable contribution on the two main PCA axes with the percentage of variance explained in each axis; (**b**) individual contribution of each environmental variable to first PCA axis and (**c**) individual contribution of each environmental variable to the second PCA axis. Variable names in x-axis correspond to: bio3 = isothermality, bio4 = temperature seasonality, bio8 = mean temperature of wettest quarter, bio10 = mean temperature of warmest quarter, bio11 = mean temperature of coldest quarter, bio15 = precipitation seasonality, bio16 = precipitation of wettest quarter, EVI mean = mean of primary productivity and EVI seasonality = primary productivity seasonality.

Appendix 3.3 Environmental gradients of the Iberian Peninsula

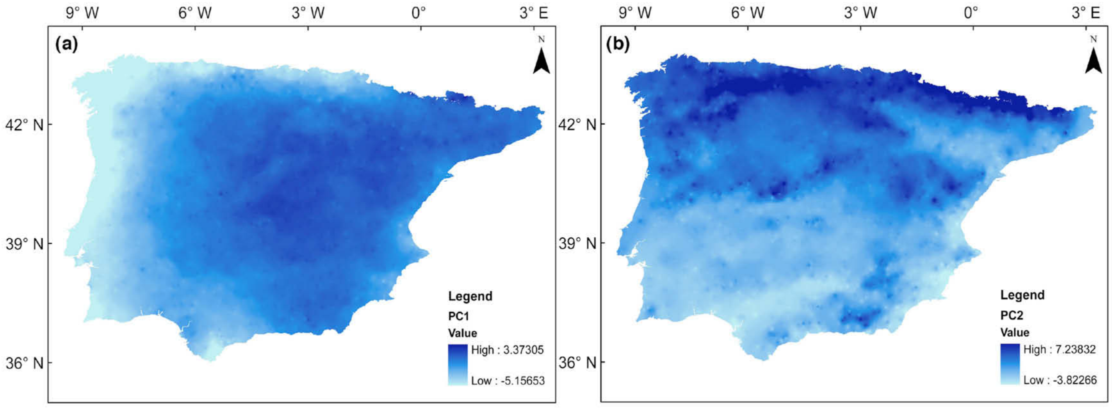

Figure A3.3 Environmental gradients of the Iberian Peninsula depicted by (**a**) the first axis and (**b**) second axis of the environmental Principal Component Analysis (PCA). Higher intensity in the blue scale represents higher values, while smaller intensity represent lower values. PCA was built with climatic and primary productivity variables (see Chapter III MATERIAL AND METHODS for a detailed description).

Appendix 3.4 Mapping PCA scores for the two first axes from the env-PCA

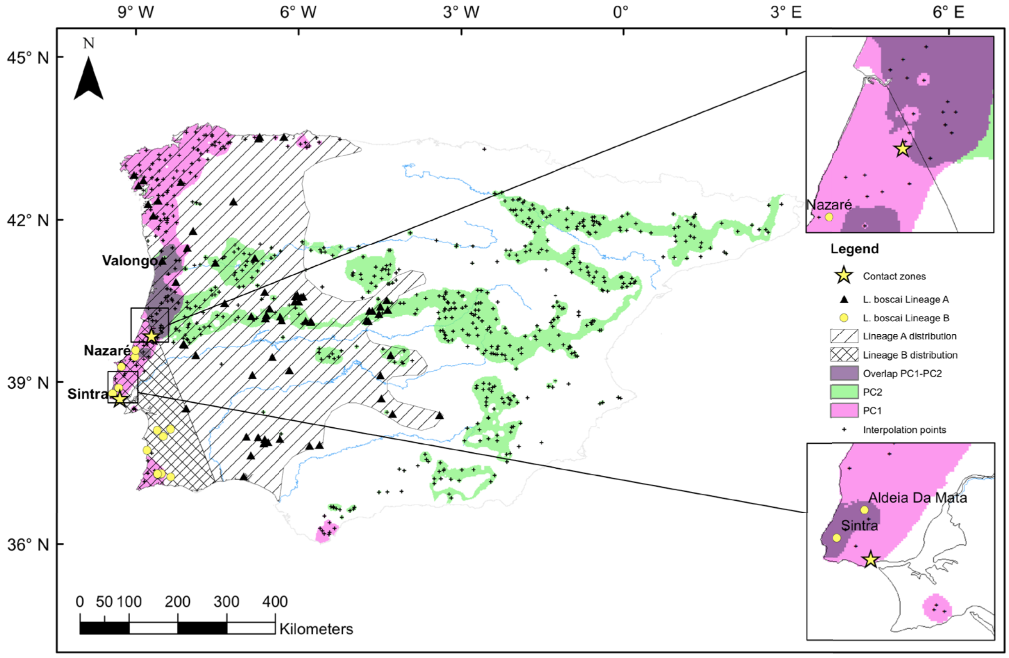

Figure A3.4 PCA scores for the first two axes retrieved by the environmental-PCA mapped within the geographical context of the Iberian Peninsula. The presence records for lineage A are shown as black triangles, and for lineage B are shown as yellow circles. The simple hatched area represents distributional range for lineage A and the cross hatched area represents distributional range for lineage B. PC1 is colored in pink, PC2 in green, and the overlap between PC1 and PC2 in purple. Stars show localities where second contacts between lineages A and B exist (see Fig. 1a in Teixeira et al., 2015). Background points used for interpolation are shown as black crosses. Niche overlap areas with contact zones are enlarged in separate panels.

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