

## Biometrical analysis reveals major differences between the two subspecies of the European rabbit

CATARINA CAMPOS FERREIRA<sup>1,2,3\*</sup>, FRANCISCA CASTRO<sup>4,5</sup>, VICENTE PIORNO<sup>6</sup>, ISABEL CATALÁN BARRIO<sup>7,8</sup>, MIGUEL DELIBES-MATEOS<sup>1,2,4</sup>, CARLOS ROUCO<sup>9</sup>, LUIS E. MÍNGUEZ<sup>1</sup>, FERNANDO APARICIO<sup>4,5</sup>, JOSÉ A. BLANCO-AGUIAR<sup>1,2</sup>, ESTHER RAMÍREZ<sup>1</sup>, CANDELARIA IRIARTE<sup>1</sup>, CARLOS A. RÍOS-SALDAÑA<sup>10</sup>, JESÚS CAÑADILLA<sup>1</sup>, LUIS ARIAS DE REYNA<sup>5</sup>, PABLO FERRERAS<sup>1</sup>, PAULO C. ALVES<sup>2,11,12</sup> and RAFAEL VILLAFUERTE<sup>4,5</sup>

<sup>1</sup>*Instituto de Investigación en Recursos Cinegéticos (IREC-CSIC-UCLM-JCCM), Ronda de Toledo, s/n, 13071, Ciudad Real, Spain*

<sup>2</sup>*InBio Laboratório Associado, CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus Agrário de Vairão, 4485-661, Vairão, Portugal*

<sup>3</sup>*Department of Biology, Trent University, Peterborough, ON, K9J 7B8, Canada*

<sup>4</sup>*Instituto de Estudios Sociales Avanzados (IESA-CSIC), Campo Santo de los Mártires, 14004, Córdoba, Spain*

<sup>5</sup>*Dpto. Zoología, Universidad de Córdoba, Campus de Rabanales, 14071, Córdoba, Spain*

<sup>6</sup>*Parque Nacional de las Islas Atlánticas de Galicia, Calle Oliva, 3, 36202, Vigo, Spain*

<sup>7</sup>*Department of Biological Sciences, University of Alberta, Biological Sciences Building CW-405, Edmonton, AB, T6G2E9, Canada*

<sup>8</sup>*Pyrenean Institute of Ecology (CSIC), Avda. Nuestra Señora de la Victoria, 22700, Jaca, Spain*

<sup>9</sup>*Landcare Research, PO Box 1930, 9054, Dunedin, New Zealand*

<sup>10</sup>*BioCórima A. C., Blvd. Dr. Jesús Valdés Sánchez km 10, Col. Presa de las Casas, Arteaga, Coahuila, 25350, México*

<sup>11</sup>*Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, s/n, Edifício FC4, 4169-007, Porto, Portugal*

<sup>12</sup>*Wildlife Biology Program, College of Forestry and Conservation, University of Montana, Missoula, MT, USA*

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The climatic oscillations that have occurred in the last few million years have strongly affected species distribution ranges. Highly divergent genetic lineages arose, some of which correspond to recognized subspecies that currently occupy small geographical areas. Understanding the implications of the genetic differences between these subspecies is crucial for proper conservation of Evolutionarily Significant Units. We use the two European rabbit subspecies, *Oryctolagus cuniculus cuniculus* and *O. c. algirus*, in the Iberian Peninsula as a model to investigate the repercussions at the biometric level of their largely recognized genetic differentiation. To accomplish this we analysed the ear and hind foot length, and the body mass of 999 adult rabbits from 27 locations across the distribution range of both subspecies in their native range, the Iberian Peninsula. Our results show biometric differences between the two subspecies, also explained by geographical location and sex, *O. c. algirus* being lighter and having shorter ear and hind foot lengths. We examine these findings under an evolutionary framework, and discuss their implications for current conservation efforts. Future research should focus on the ecological implications of these biometric differences, namely potential different habitat use and anti-predatory strategies in the species' native range. §[Corrections added on 24 February 2016, after issue publication: The Acknowledgements section has been updated to

\*Corresponding author. E-mail: catferreira@gmail.com

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## INTRODUCTION

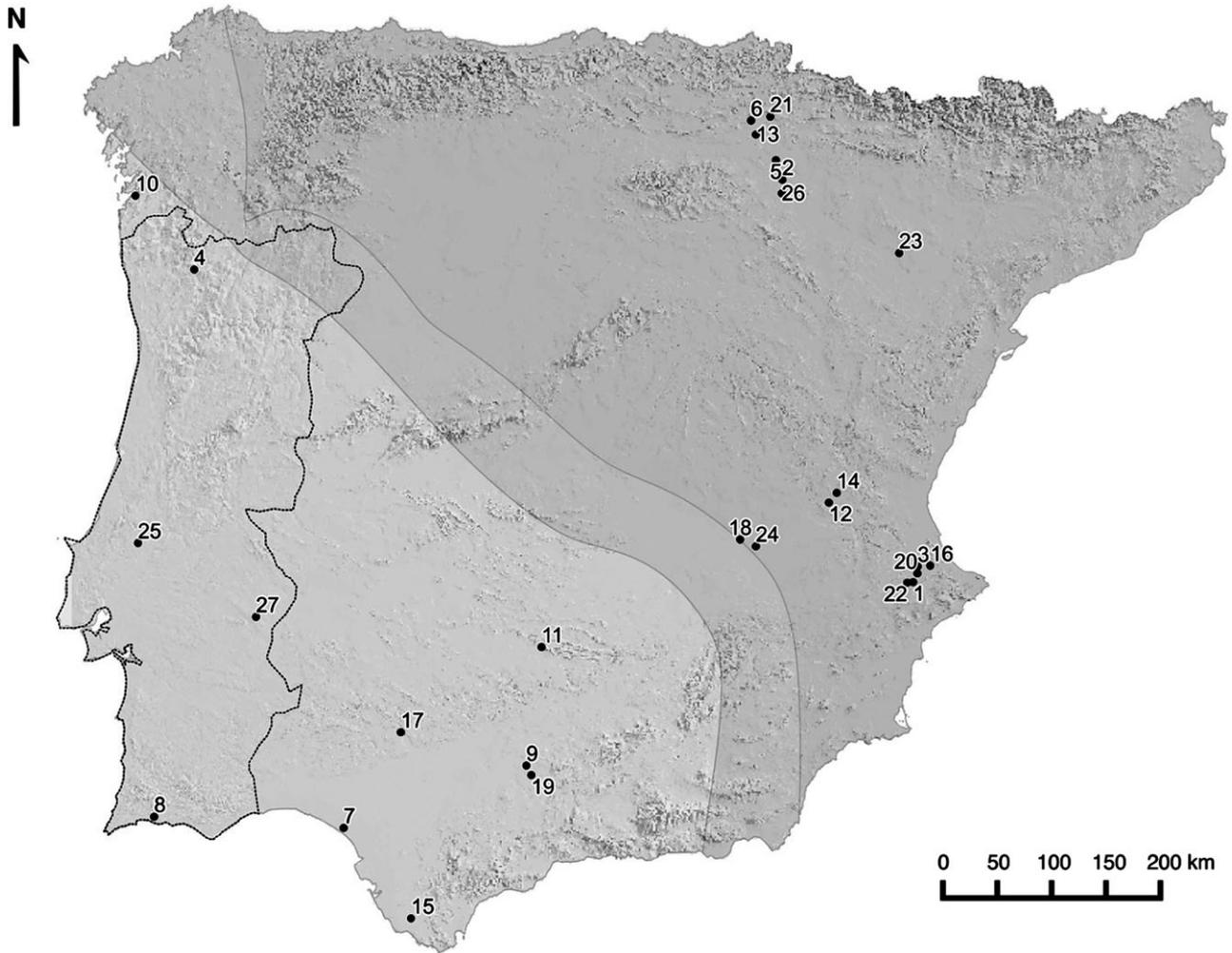
The climatic oscillations that have occurred in the last few million years are thought to have strongly affected the distribution ranges of species (Hewitt, 2000). In particular, Pleistocene climatic modifications were responsible for major changes in the location of refugia in southern Eurasia, which probably hosted temperate species during the last glaciations (Randi, 2007). The Iberian, Italian and Balkan peninsulas have been identified as the three main glacial refuge areas during the Pleistocene in Europe (Randi, 2007), acting as ‘hotspots’ of endemism and catalysts of speciation mechanisms as the result of geographical isolation (Bilton *et al.*, 1998). This has given rise to highly divergent genetic lineages in several species, some of which correspond to recognized subspecies that currently occupy small geographical areas (Weiss & Ferrand, 2007). Maintaining the integrity of these lineages by conserving this high genetic diversity is imperative to deter ongoing biodiversity loss (O’Brien & Mayr, 1991; Hey *et al.*, 2003).

The European rabbit (*Oryctolagus cuniculus* Linnaeus, 1758) is a native species from the Iberian Peninsula (Ferrand, 2008), which was historically very abundant. The species is the sole representative of its genus and it is considered a keystone species in Iberian Mediterranean ecosystems, particularly for its importance in the trophic ecology of these communities and its role as an ecosystem engineer (reviewed by Delibes-Mateos *et al.*, 2008a). Molecular data have recurrently confirmed the existence of two divergent rabbit groups (Biju-Duval *et al.*, 1991; Branco, Ferrand & Monnerot, 2000; Carneiro *et al.*, 2013, 2014), corresponding to the two known subspecies: *O. c. cuniculus* and *O. c. algirus*. The divergence between the two rabbit subspecies, detected at multiple loci (e.g. Geraldès, Ferrand & Nachman, 2006; Carneiro *et al.*, 2010, 2013) and at the genomic level (Carneiro *et al.*, 2014), suggests that they have evolved independently for a long time, after splitting approximately 2 Mya during the Quaternary glaciations (Ferrand, 2008). During this period, both subspecies remained in allopatry in two glacial refugia (Branco *et al.*, 2002), one located in the southernmost tip of Iberia and the other in the eastern Mediterranean coast of Spain and the Ebro valley (Ferrand, 2008). After glaciations, rabbit populations

from the two subspecies expanded their ranges, tracking the amelioration of climatic conditions from both the southwestern and the eastern refuges in the direction of central Iberia (Branco *et al.*, 2002). Currently, the two rabbit subspecies are distributed in parapatry in the central part of the Iberian Peninsula, where they also hybridize (Branco *et al.*, 2000, 2002; Geraldès *et al.*, 2006, 2008; Carneiro, Ferrand & Nachman, 2009; Carneiro *et al.*, 2010, 2013; Fig. 1). Despite the existence of a postglacial contact zone (Branco *et al.*, 2000, 2002) the subspecies *algirus* is mainly restricted to the western portion of the Iberian Peninsula whereas the subspecies *cuniculus* is distributed towards the north-east (Fig. 1).

The intraspecific taxonomy of the European rabbit is supported not only genetically, but also by parasitological (Launay & Beaucournu, 1982; Beaucournu & Marquez, 1987; Beaucournu & Launay, 1990) and reproductive studies (Gonçalves, Alves & Rocha, 2002). However, morphological evidence is quite scarce and ambiguous. For example, Sharples, Fa & Bell (1996) found only slight differences in cranial measurements between the two subspecies that they considered to be insufficient to separate the European rabbit into distinct groups. Conversely, Villafuerte (2002) described that the asymptotic body mass was largely non-overlapping between the two subspecies with *O. c. cuniculus* being heavier than *O. c. algirus*. Nevertheless, the demonstration of such morphological differences between rabbit subspecies has relied on only a small sample of Iberian populations that included individuals not genetically validated as belonging to one or the other subspecies.

Identifying units below the species level that differ in their genetic and ecological characteristics is of great importance to understand their biology and to adjust the corresponding conservation or management actions. One common approach in this respect is the definition of Evolutionarily Significant Units (ESUs; Moritz, 1999), of populations that should be managed separately to prioritize conservation efforts. In genetic terms, ESUs have been defined as populations that have substantial reproductive isolation which has led to adaptive differences. However, it has been suggested that ecological differentiation should also be taken into account when defining



**Figure 1.** Distribution of European rabbit biometric data collected in the Iberian Peninsula, from 1989 to 2011, according to the proposed division of subspecies (light grey *O. c. algirus*, secondary contact zone and dark grey *O. c. cuniculus*; Carneiro *et al.*, 2013). Note: numbers correspond to populations (Province), as follows: 1, Agullent (Valencia); 2, Arguedas (Navarra); 3, Bellús (Valencia); 4, Cabreira (Vieira do Minho); 5, Caparroso (Navarra); 6, Cirauqui (Navarra); 7, L'Algaida, Doñana (Huelva); 8, Estômbar (Portimão); 9, Aguilar de la Frontera (Córdoba); 10, Vigo (Galicia); 11, La Garganta (Ciudad Real); 12, Jaraguas (Valencia); 13, Larraga (Navarra); 14, Los Corrales (Valencia); 15, Las Lomas (Cádiz); 16, Lucient (Valencia); 17, Melonares (Sevilla); 18, Minaya (Albacete); 19, Moriles (Córdoba); 20, Montaverner (Valencia); 21, Navarra (Navarra); 22, Ontinyent (Valencia); 23, Pina de Ebro (Zaragoza); 24, La Roda (Albacete); 25, Pernes (Santarém); 26, Tudela (Navarra); 27, Vila Viçosa (Vila Viçosa).

units of management (Crandall *et al.*, 2000). Proper delimitation of these units is of particular interest when the species or differentiated populations of the species under management are frequently translocated, due to the risks associated with the admixture of populations with different genetic characteristics and the subsequent outbreeding depression. European rabbit populations are intensively managed in the Iberian Peninsula (e.g. Ferreira *et al.*, 2014), and restocking with translocated or captive-reared individuals is a frequent strategy that has raised concerns about artificial alteration of the genetic

structure of the species (Delibes-Mateos *et al.*, 2008b). Understanding the implications of the genetic differences between rabbit subspecies is crucial for proper management of the species and the conservation of rabbit ESUs. A first step to understand the differences between rabbit subspecies is the study of possible biometric differences, as these dissimilarities may reflect adaptations to diverse environmental conditions.

The main goal of this study was to investigate if the genetic differentiation between the two European rabbit subspecies is reflected in biometric differences

within their native range, the Iberian Peninsula. Our hypothesis is that such biometric differences exist and that they can be explained by the evolutionary history of the two subspecies and their geographical distribution.

## MATERIAL AND METHODS

Data on the European rabbit's biometry were collected from 27 sites located across the species distribution range in the Iberian Peninsula (Fig. 1). Data collection spanned an interval of 22 years (1989–2011) and comprised 999 individuals in total. Over this period, rabbits were collected using both scientific captures of live rabbits with ferrets (*Mustela putorius* Linnaeus, 1758) (i.e. ferreting with nets and traps), and in the course of regular game activities (i.e. rabbits killed with shotgun by hunters during the hunting season). All animal manipulations conducted in this work complied with the standards and procedures promulgated by the National Authorities legislation. Several different biometric measurements were taken over the 22 years of data collection but for the purposes of this study we considered ear and hind foot lengths (measured according to the protocol in the Supporting Information, Fig. S1) and body mass because they were the only ones consistent across populations. Sex was also determined for each individual rabbit. Only adult rabbits were included in the analyses. Rabbits were considered to be adults when their body mass (regardless of subspecies) exceeded the weight of the lightest individual showing signs of sexual maturity (scrotal tests for males and lactating and/or pregnant females, as assessed by inspection of mammary gland development and abdominal palpation; males > 750 g and females > 900 g; Villafuerte, 1994). Pregnant and lactating adult females were also excluded from the statistical analyses of body mass, but were included in ear and hind foot length analyses. The potential for a non-detection of pregnant and/or lactating females in this study is considered very low because the method is very reliable. However, an error could have occurred for females in an early stage of pregnancy, although at this stage the variations in body mass would be negligible. We explicitly excluded populations from the known hybrid zone, as we were interested in assessing the morphological differences between the two subspecies as separate ESUs (Fig. 1). The subspecies to which each individual rabbit belonged was determined genetically for all individuals either specifically in this work or in previous studies; in all cases, this identification was

based on three diagnostic loci located on cytochrome b, on the X and Y chromosomes (Branco *et al.*, 2000; Geraldès *et al.*, 2006, 2008).

## STATISTICAL ANALYSES

Mean ( $\pm$  SD) ear length, hind foot length and body mass were estimated for each sex, population and subspecies. To analyse the variables that determine the biometric differences between subspecies we used linear mixed models (LMMs) with a normal error structure and an identity link function. We built LMMs using ear length, hind foot length and body mass as the response variables, respectively. Initial models included the effects of site latitude and longitude, sex, and subspecies (A, *O. c. algirus*; B, *O. c. cuniculus*), and the two-way interactions between latitude and longitude, latitude and subspecies, and subspecies and sex. Two-way interactions reflected our biological understanding of the system and were included to account for potential non-linear spatial patterns (latitude  $\times$  longitude), differential responses of the subspecies to north–south gradients (latitude  $\times$  subspecies), and sex-related differences between the subspecies (sex  $\times$  subspecies). In the model for ear length, the independent effect of hind foot length was also included as a predictor variable to account for potential differences in rabbit size. Site and sampling year were included as random variables to account for data structure. When testing for multicollinearity among predictor variables, we found that latitude and longitude differed between subspecies in all cases ( $P > 0.6$ ); in the model for ear length, hind foot length was also different between subspecies ( $P > 0.5$ ). To avoid problems due to collinearity (Graham, 2003), we computed a regression of subspecies against the problematic continuous variables, and in further analyses we replaced the former variables with the residuals from this regression. Having removed the variation due to subspecies, these residuals represent the unique contribution of latitude, longitude and hind foot length of subspecies-induced effects. Model selection followed a backward stepwise procedure based on Akaike's Information Criterion (AIC) and likelihood ratio tests (LRT); models with and without a certain variable were compared, and only variables that significantly improved model fit were retained in the final model for each response variable (Zuur *et al.*, 2009). All modelling assumptions, including spatial autocorrelation, were checked through analyses of residuals (Zuur *et al.*, 2009). All statistical analyses were performed using R 3.0.3 (R Core Team, 2014) and the packages *ncf* for building spatial correlograms and *nlme* for LMMs.

**Table 1.** Means  $\pm$  SD for ear length, hind foot length and body mass, and respective sex ratio (F:M) of 999 adult European rabbits belonging to each of the two subspecies (A, *Oryctolagus cuniculus algirus*; B, *O. c. cuniculus*) from 27 sites sampled across the Iberian Peninsula

Biometry	Sub	Adult males	<i>n</i>	Adult females	<i>n</i>	All adults	<i>N</i>	F:M
Ear length (mm)	A	72.62 $\pm$ 5.71	448	72.95 $\pm$ 5.82	304	72.76 $\pm$ 5.75	752	1.00
	B	79.10 $\pm$ 4.74	117	78.38 $\pm$ 4.89	130	78.72 $\pm$ 4.82	247	0.99
Hind foot length (mm)	A	56.22 $\pm$ 3.47	448	56.61 $\pm$ 3.34	304	56.38 $\pm$ 3.42	752	1.00
	B	59.58 $\pm$ 3.78	117	59.34 $\pm$ 2.71	130	59.45 $\pm$ 3.26	247	0.99
Body mass (g)	A	1003.17 $\pm$ 120.28	448	1102.41 $\pm$ 139.62	304	1043.29 $\pm$ 137.30	752	1.10
	B	1208.67 $\pm$ 159.76	117	1257.74 $\pm$ 174.89	130	1234.49 $\pm$ 169.34	247	1.04

## RESULTS

Of the 999 rabbits analysed, 752 belonged to the subspecies *O. c. algirus* and 247 to *O. c. cuniculus*. Results showed marked biometric differences between the two subspecies (Table 1; for results per population and individual sex see Table S1). Overall, ear length (mean  $\pm$  SD) was shorter for adult *O. c. algirus* than for adult *O. c. cuniculus* (*algirus* = 72.76  $\pm$  5.75 mm, *cuniculus* = 78.72  $\pm$  4.82 mm), and hind foot length followed the same pattern (*algirus* = 56.38  $\pm$  3.42 mm, *cuniculus* = 59.45  $\pm$  3.26 mm; Table 1). Body mass was consistent with this trend, with *algirus* rabbits being lighter than *cuniculus* (*algirus* = 1043.29  $\pm$  137.30 g, *cuniculus* = 1234.49  $\pm$  169.34 g; Table 1).

The final model for ear length included the independent effect of latitude, longitude, subspecies and hind foot length as significant predictors (Table 2). For hind foot length, latitude and the two-way interaction between sex and subspecies were retained (Table 2, Fig. 2). Longitude had a positive effect on ear length and body mass, indicating that ears were longer and rabbits were slightly heavier towards the eastern part of the study area. Subspecies had a positive effect on ear, hind foot length and body mass, indicating greater sizes for the subspecies *O. c. cuniculus*. For latitude, opposing patterns were found for ear and hind foot lengths; ears tended to be shorter and feet to be longer at the northernmost populations. Finally, the two-way interaction between sex and subspecies explained differences in hind foot length and body mass, which indicated that the difference in size between males and females was smaller in the subspecies *O. c. cuniculus* (Table 2, Fig. 2). Notwithstanding, for hind foot length this difference between sexes was not significant (Table 2).

## DISCUSSION

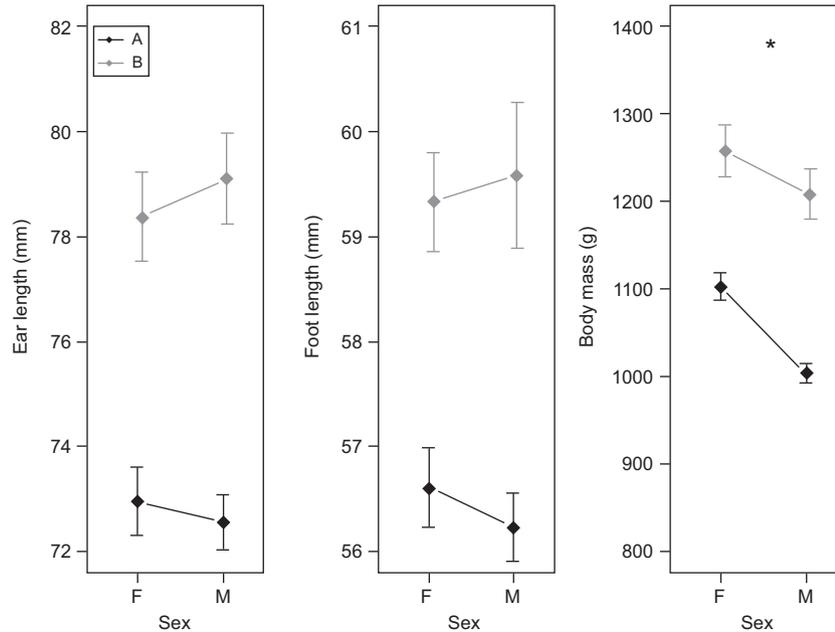
To our knowledge, this is the first study performed at a large scale on the biometry of the European rab-

**Table 2.** Results for the LMMs of each biometric parameter considered in this study based on 999 European rabbits from 27 sites sampled across the Iberian Peninsula

	Estimate $\pm$ SE	d.f.	<i>t</i> -value	<i>P</i>
<b>Ear length</b>				
Intercept	74.00 $\pm$ 0.77	957	95.41	< 0.001
Longitude <sup>R</sup>	1.41 $\pm$ 0.24	957	5.97	< 0.001
Latitude <sup>R</sup>	-0.95 $\pm$ 0.29	957	-3.19	0.001
Subspecies (B)	4.83 $\pm$ 1.08	957	4.46	< 0.001
Foot length <sup>R</sup>	0.34 $\pm$ 0.04	957	7.48	< 0.001
<b>Hind foot length</b>				
Intercept	57.14 $\pm$ 0.50	958	113.44	< 0.001
Latitude <sup>R</sup>	0.60 $\pm$ 0.21	958	2.90	0.0038
Subspecies (B)	2.04 $\pm$ 0.65	958	3.12	0.0019
Sex (M)	-0.28 $\pm$ 0.18	958	-1.49	0.1373
Sub $\times$ sex (B $\times$ M)	0.71 $\pm$ 0.37	958	1.91	0.0568
<b>Body mass</b>				
Intercept	1100.68 $\pm$ 26.11	958	42.15	< 0.001
Longitude <sup>R</sup>	16.28 $\pm$ 7.91	958	2.06	0.0399
Subspecies (B)	160.06 $\pm$ 34.03	958	4.70	< 0.001
Sex (M)	-85.29 $\pm$ 9.38	958	-9.09	< 0.001
Sub $\times$ sex (B $\times$ M)	44.50 $\pm$ 18.90	958	2.35	0.0187

Superscript R indicates variables for which residuals of the regression against subspecies were taken (see text for details). For categorical variables the level tested against the baseline is indicated in parentheses (B, *Oryctolagus cuniculus cuniculus*; M, males).

bit subspecies in its native range, the Iberian Peninsula, using genetic validation. Our results suggest that there are unequivocal biometric differences between rabbit subspecies. Rabbits from the *O. c. algirus* populations have smaller ears (even when controlling for the effect of body mass, i.e. hind foot length) and feet in relation to the *O. c. cuniculus* populations, and they are also lighter.



**Figure 2.** Effect of the interactions between sex (M, male; F, female) and subspecies (A, *Oryctolagus cuniculus algirus*; B, *O. c. cuniculus*) in ear and hind foot lengths and body mass for 999 adult European rabbits from 27 sites sampled across the Iberian Peninsula. Mean values and 95% confidence intervals are shown. An asterisk indicates a significant interaction ( $P < 0.05$ ).

Dissimilar inter-sexual non-significant trends between rabbit subspecies were also observed, with *O. c. algirus* males having smaller ears and feet than *O. c. algirus* females, and *O. c. cuniculus* males showing the opposite pattern (Fig. 2). This could be related to the fact that *O. c. algirus* reaches sexual maturity at an earlier age (and smaller body mass) than *O. c. cuniculus* as a result of living under different climatic conditions (Gonçalves *et al.*, 2002). Another explanation could be related to differences in the composition and abundance of the predator communities across the Iberian Peninsula (Mathias *et al.*, 1998; Martí & Del Moral, 2003; Palomo, Gisbert & Blanco, 2007), and thus in predation risk, which would probably affect males more than females (Villafuerte, 1994). Nevertheless, these differences were not significant, and therefore caution should be given to these considerations.

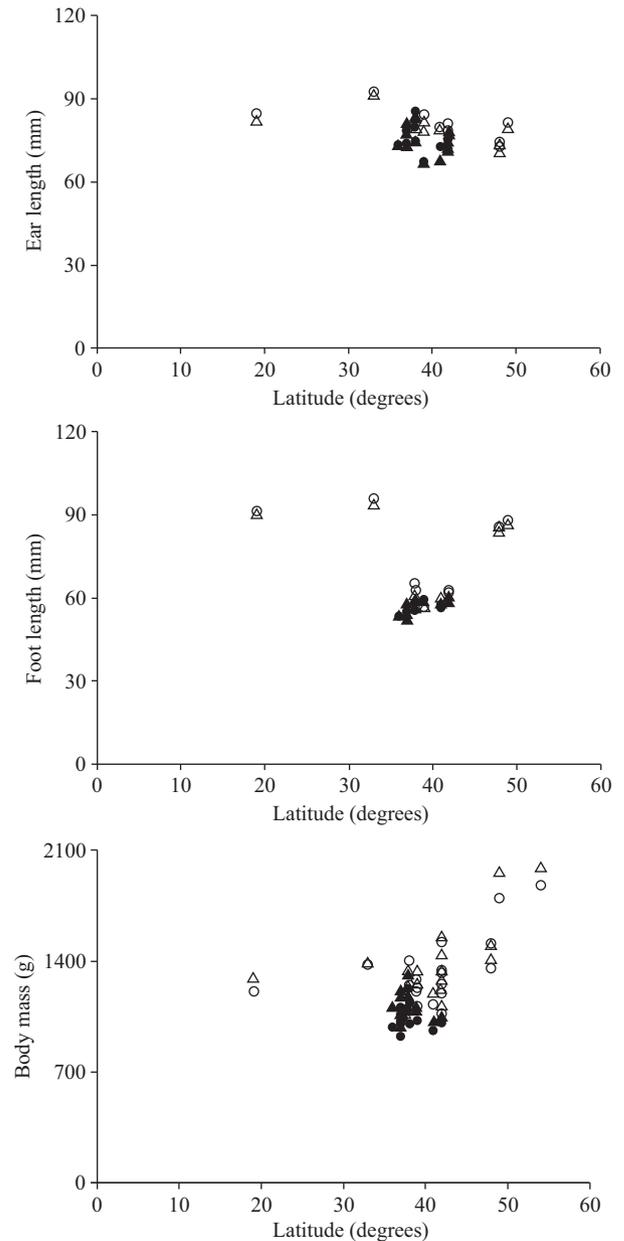
For body mass, a two-way interaction between subspecies (*O. c. cuniculus*) and sex (males) explained part of the variation found in the models, suggesting that there are differences between sexes and subspecies when considering body size. Our results suggest the occurrence of reversed sexual dimorphism (RSD) in both European rabbit subspecies, as females were slightly heavier than males. Furthermore, *O. c. algirus*, the lighter subspecies, showed higher RSD than *O. c. cuniculus* (Table 1), in accordance with Rensch's rule that predicts that within a lineage size

dimorphism will decrease with increasing body size when the female is the larger sex (Rensch, 1950; Fairbairn, 1997). Sexual dimorphism is a pattern seen throughout the animal kingdom and is exhibited in a myriad of ways (e.g. coloration, body size). Because of their different roles in reproduction, males and females are often under selection that favours their divergent morphological appearance (Badyaev, 2002), and in mammals this is typically biased towards males (Davis & Roth, 2008). Nevertheless, RSD seems to be a common pattern in lagomorphs, also reported in *Sylvilagus* rabbits and in hares (genus *Lepus*), as well as in domestic rabbits (Davis & Roth, 2008; Alves, Ferrand & Hackländer, 2010; Rosell & de la Fuente, 2012). Larger size may be advantageous in terms of behaviour and dominance for access to a partner, or to resources, but also in terms of foraging, nest protection, breeding investment and energetics or a combination thereof (Shine, 1989). Larger female size may be related to high fecundity (as seen in the European rabbit for the *O. c. cuniculus* subspecies; Gonçalves *et al.*, 2002), or reduced nest mortality (Rödel *et al.*, 2009), a suite of traits (female dispersion, large male home-ranges, reduced aggression and a promiscuous mating system) have been proposed to favour smaller males in *Sylvilagus* rabbits (Davis & Roth, 2008). In the European rabbit, there are no apparent differences in mating system or foraging strategies (eco-

logical specialization) that could explain the differences in size dimorphism between the two subspecies, and so the impact of these potential mechanisms requires further investigation.

An interesting observation in our study was that ear length, although generally smaller than for rabbit populations outside Iberia, was still larger in southern Iberian populations when controlling for the effect of subspecies and body mass (as suggested by a negative coefficient for latitude in the ear length model, Table 2). A potential explanation for this could be thermoregulation, as southern Iberia is under a Mediterranean semi-arid climate with mean annual maximum temperatures of 24 °C (Ninyerola, Pons & Roure, 2005). This phenomenon has been described also for several mammals (e.g. Lindstedt & Boyce, 1985) including other lagomorphs (e.g. genus *Lepus*, Stott, Jennings & Harris, 2010). Moreover, studies from Australia have shown that European rabbits introduced in a warmer environment responded by increasing the mean length of their ears (Parer & Libke, 1985). Bigger ears could provide an advantage to facilitate heat interchange (i.e. Allen's rule), probably more as a consequence of phenotypic than genetic variation (Serrat, King & Lovejoy, 2008). Nevertheless, experiments by Williams & Moore (1989, 1990) showed that although much of the variability between locations is phenotypic adaptation, there is a small, detectable genetic component of differences in ear size relative to body size. These authors argued that the growth of the extremities is not just determined by ambient temperature but also by the number of littermates. Likewise, pinnae and other organs with a thermoregulatory function are influenced by temperature and season of birth, i.e. rabbits that matured during winter could have smaller ears than those that grew in late spring and early summer. Unfortunately, our dataset did not allow for such finer-scale analyses, although we acknowledge that both season of birth and number of littermates could play a role in the variation detected between the two rabbit subspecies across the Iberian Peninsula.

Previous work on the differentiation of rabbit subspecies in Europe has relied largely on differences in either body mass (Soriguer, 1980; Villafuerte, 2002) or pelage (Callou, 2002), although in some of these studies not all rabbits were subjected to a genetic assignment of the subspecies. These variations have been attributed mainly to latitudinal effects (Callou, 2002), as the pattern is observed similarly in rabbit populations across the globe (although outside Iberia these are represented only by *O. c. cuniculus*), where an increase in body mass (and ear length) with increasing latitude is evident for both males and females, supporting Bergmann's rule (interestingly,



**Figure 3.** Correlation between average biometric variables (ear and hind foot lengths, and body mass) of populations of *Oryctolagus cuniculus algirus* and *O. c. cuniculus* and absolute latitude worldwide. Circles represent males and triangles represent females; closed circles and triangles denote *O. c. algirus* and open circles and triangles denote *O. c. cuniculus*. Data comprise the 27 populations included in this study with additional data collected from the following studies: Boussett (1991), Lopes Ribeiro (1981), Merton (1987), Rogers, Arthur & Soriguer (1994), Skira (1980) and Zunino (1989).

the pattern is not as clear for ear length; Fig. 3). Our results also support this evidence, as site latitude and longitude were retained in our final model

for ear length, latitude for ear length and longitude for body mass. Nevertheless, the effect of subspecies was clearly stronger for all biometric variables (Table 2). These biometric differences could translate into ecological particularities of each subspecies and there is some evidence to support this. For example, since the beginning of the 20th century, the two subspecies were thought to exhibit distinct characteristics, with *O. c. cuniculus* usually being described as 'the larger and perhaps hardier breed' (Cabrera, 1914), which 'displayed remarkable adaptability to extreme conditions' (Gibb, 1990). These observations were further corroborated by Cockerell (1921) who drew attention to similarities between the Porto Santo rabbit and the southern European subspecies of *O. cuniculus*, advocating that they were the same and that both were different from the English rabbit, contrary to what had been proposed initially by Darwin. On the other hand, there is strong evidence for reproductive differences between the two subspecies with lower breeding parameters (e.g. average litter size) for the *O. c. algirus* subspecies (Gonçalves *et al.*, 2002). Osteometric and osteoscopic analyses have also shown obvious differences between the two subspecies (cranial and post-skeletal features smaller in *O. c. algirus*), as the result of a morphological evolution in function of their geographical position (latitude) and their environment (Callou, 2002). Therefore, the results of our study are in clear accordance with these findings, as ear and hind foot lengths, and body mass are also different between the two subspecies, within the species native range, with values lower for *O. c. algirus*. If the differences in body size offer some reproductive advantage, they might promote not only hybridization in the secondary contact zone but also reproductive asymmetry. Despite the detection of hybrids in the wild, molecular studies have not shown the existence of asymmetric gene flow (e.g. Carneiro *et al.*, 2014), suggesting that body size is probably not playing an important role in sexual selection, and some other behavioral mechanism (e.g. assortative mating) may be working to prevent directional mixing.

Several cases described in the literature support the idea that animal populations that have been historically geographically isolated frequently undergo speciation and can generate different species (Coyne & Orr, 2004). The ultimate differences can manifest not only at the genetic level but also morphologically [e.g. European lynx (*Lynx lynx* Linnaeus, 1758) and the Iberian lynx (*Lynx pardinus* Temminck, 1824), Ferrer & Negro, 2004]. The fossil record of *O. cuniculus* indicates that, at least in south-western Spain, in the area of distribution of *O. c. algirus*, the rabbit has undergone a marked reduction in body size during the last 5000 years (Bernáldez-Sánchez, Bernál-

dez-Sánchez & Viñas, 2013). The causes for this reduction in body size, also observed in wild ungulates, are not clear but may be due to climatic factors or anthropogenic influence (Bernáldez-Sánchez *et al.*, 2013), which could have favored the morphological differentiation between subspecies. A more recent form of anthropogenic influence, e.g. through artificial selection, could also have played a role in this differentiation. Rabbit domestication was initiated approximately 1400 years ago in France, mainly to obtain meat and fur (Clutton-Brock, 1999). The larger size and productivity of the subspecies *O. c. cuniculus* probably favoured its selection for controlled breeding. Our results suggest that 2 Myr of independent evolutionary histories may have prompted the development of the biometric differences observed between the two European rabbit subspecies. The latter reinforces the distinctiveness of *O. c. cuniculus* and *O. c. algirus* and highlight the need for a differential assessment of the two rabbit subspecies. This can be particularly crucial for *O. c. algirus*, which is restricted to the south-western portion of the Iberian Peninsula (Ferrand, 2008) and presents an exceptionally high nucleotide diversity in comparison with the *O. c. cuniculus* populations both in Iberia and worldwide, probably as a result of the influence of historical biogeographical events (e.g. glaciations) on Iberian biota (Carneiro *et al.*, 2009). The best way to preserve this genetic diversity, which is mirrored in the biometric differences found, would be their recognition at least as two different ESUs, and to perform distinct threat assessments using IUCN criteria, thereby ensuring the protection of the idiosyncrasies of each rabbit subspecies. A similar approach has been used for a roe deer (*Capreolus capreolus* Linnaeus, 1758) population isolated in southern Spain, which shows biometric differences with respect to other populations within the species range, and is currently the target of specific conservation efforts (Aragón, Braza & San José, 1998; San José, Fernández-Salguero & Redondo, 2013). Our results could also be useful to prevent the artificial admixture of rabbit subspecies caused by restocking operations through, for instance, the development of alternative methods of differentiating both rabbit subspecies in the field. Nevertheless, accurate subspecies assignment should rely on genetic analysis, as fine-tuning of alternative tools still requires further research.

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## SUPPORTING INFORMATION

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

**Figure S1.** Protocol for biometric measures in the wild rabbit: A, ear length; B, hind foot length. Drawings by J. López-Rojas.

**Table S1.** Ear, and hind foot lengths and body mass (mean  $\pm$  SD) of 999 adult male and female rabbits from 27 sites sampled across the Iberian Peninsula. NA represents populations where  $n = 1$  and hence SD could not be calculated.