Zoology 118 (2015) 377–385

Contents lists available at ScienceDirect

Zoology

journal homepage: www.elsevier.com/locate/zool

Strong spatial segregation between wildcats and domestic cats may explain low hybridization rates on the Iberian Peninsula

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ARTICLE INFO

Article history:
Received 10 February 2015
Received in revised form 18 May 2015
Accepted 6 August 2015
Available online 11 August 2015

Keywords:
Ecological barriers
Felis silvestris
Genetic introgression
Interspecific hybridization
Wildcat conservation

ABSTRACT

The European wildcat (*Felis silvestris silvestris*) is an endangered felid impacted by genetic introgression with the domestic cat (*Felis silvestris catus*). The problem of hybridization has had different effects in different areas. In non-Mediterranean regions pure forms of wildcats became almost extinct, while in Mediterranean regions genetic introgression is a rare phenomenon. The study of the potential factors that prevent the gene flow in areas of lower hybridization may be key to wildcat conservation. We studied the population size and spatial segregation of wildcats and domestic cats in a typical Mediterranean area of ancient sympatry, where no evidence of hybridization had been detected by genetic studies. Camera trapping of wild-living cats and walking surveys of stray cats in villages were used for capture–recapture estimations of abundance and spatial segregation. Results showed (i) a low density of wildcats and no apparent presence of putative hybrids; (ii) a very low abundance of feral cats in spite of the widespread and large population sources of domestic cats inhabiting villages; (iii) strong spatial segregation between wildcats and domestic/feral cats; and (iv) no relationship between the size of the potential population sources and the abundance of feral cats. Hence, domestic cats were limited in their ability to become integrated into the local habitat of wildcats. Ecological barriers (habitat preferences, food limitations, intra-specific and intra-guild competition, predation) may explain the severe divergences of hybridization impact observed at a biogeographic level. This has a direct effect on key conservation strategies for wildcats (i.e., control of domestic cats).

1. Introduction

Genetic introgression is a threat to the integrity of a number of animal and plant species, with human introductions of exotic species being a major contributor to this conservation problem (Rhymer and Simberloff, 1996; Ellestrand et al., 1999). A well-known example is the European wildcat (*Felis silvestris silvestris*), an endangered felid affected by introgression with domestic cat (*Felis silvestris catus*) alleles (Lozano and Malo, 2010). Wildcats and domestic cats have inhabited Europe for at least two millennia, since the introduction and spread of the latter via the expansion of the Roman Empire (Sunquist and Sunquist, 2002). However, the hybridization problem has resulted in quite different scenarios across Europe, from areas where pure forms of wildcat have become almost extinct (Hubbard et al., 1992; Beaumont et al., 2001; Daniels et al., 2001; Pierpaoli et al., 2003; Lecis et al., 2006; Hertwig et al., 2009) to areas where genetic introgression with domestic cats appears to be minimal (Randi et al., 2001; Pierpaoli et al., 2003; Lecis et al., 2006; Oliveira et al., 2008a,b; Mattucci et al., 2013; Witzenberger and Hochkirch, 2014). This has led to some controversy on the importance of the various conservation problems of the wildcat, thus hampering the design of global conservation strategies (Lozano and Malo, 2012).

Both environmental and behavioral factors may affect the degree of crossbreeding between wild cats and domestic cats. Some studies have shown a high dependence of domestic cats on humans, usually living close to inhabited country houses, a situation that may be one of the most important barriers to interbreeding (Germain, 2008; Ferreira, 2010). However, this cannot completely explain the occurrence of areas where genetic introgression is very high (Beaumont et al., 2001; Daniels et al., 2001; Pierpaoli et al., 2003; Lecis et al., 2006). Moreover, human-independent feral populations of domestic cats have been studied (Liberg, 1980; Sunquist, and Sunquist, 2002), including those inhabiting former ranges of

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http://dx.doi.org/10.1016/j.zool.2015.08.001
0944-2006/

wildcats (Zuberogoitia et al., 2001; Sarmento et al., 2009). The population size of the two sub-species in areas of co-existence is another parameter that can strongly affect gene flow, although this has not yet been sufficiently investigated.

For Mediterranean areas of the Iberian Peninsula, morphologic (Fernández et al., 1992) and molecular studies (Oliveira et al., 2008a,b) have shown low levels of genetic introgression of domestic cats in wildcat populations. For the present study, we have taken advantage of one genetically studied population of wildcats in south-eastern Spain with no evidence of domestic cat introgression (Oliveira et al., 2008b), in spite of the fact that it inhabits an area where both sub-species have co-existed for several centuries. This is an eco-demographic scenario that may be representative of large areas of the Mediterranean mountains of the Iberian Peninsula, where one of the larger pure populations of European wildcats (if not the largest) still survives (Lozano and Malo, 2012). In the present study, we seek to: (i) determine the population sizes of wildcat and domestic cat in this sympatric area; and (ii) examine the spatial segregation between them. To ascertain the reasons for the genetic integrity of the wildcats, we used the results of this investigation to test whether: (i) there are low densities of wild-living domestic cats and/or the spatial segregation is high; and whether (ii) the proximity of large population sources of domestic cats, such as villages, does not necessarily imply their colonization of wild areas. The final aim is to provide a better understanding of the hybridization problem of the threatened European wildcat and thereby help resolve a key issue regarding the proposed management measures related to the active control of the feral domestic cat (Macdonald et al., 2010; Lozano and Malo, 2012).

2. Materials and methods

2.1. Study area

The study area was the Sierra Nevada National Park and Natural Park (171.984 ha; Fig. 1). With an altitude range of 270–3492 m a.s.l., the Sierra Nevada extends axially some 100 km and has some 50 radial valleys. Optimal landscapes for wildcats (Moleón and Gil-Sánchez, 2003) are woodlands of both autochthonous oak forests (*Quercus ilex* and *Quercus pyrenaica*) and pine plantations (*Pinus pinaster*, *Pinus nigra* and *Pinus sylvestris*), which appear as pure and mixed formations in a discontinuous belt below 2400 m a.s.l. (Fig. 1). A monitoring program of the carnivorous mammals of the Sierra Nevada showed that wildcat sightings ($n = 63$) were located within these forests ($n = 60$) or very close to them ($n = 3$ at <0.25 km). These sightings were opportunistically obtained between 1990 and 2014 by field biologists, naturalists and the wildlife guards of the National Park, who work throughout the Sierra Nevada and, hence, sampling efforts could be assumed to be well distributed across the main habitats. Villages ($n = 55$) are usually small in size ($0.46 \pm 0.20\ km^2$, mean ± standard error) and located at the mouth of the valleys just outside the border of the National Park (Fig. 1). The mean distance of the villages to the nearest forest patch is 0.52 ± 0.07 km. Isolated country houses were widespread until the mid-20th century, but habitation has become rare. The Sierra Nevada, under legal protection as a Natural Park since 1989 and as a National Park since 1999, is also designated as a UNESCO Biosphere Reserve (since 1986), and as a Natura 2000 network site (since 2006).

2.2. Field survey design

The population size of wildcats and feral domestic cats was studied by non-invasive capture–recapture analysis (Long et al., 2008; O'Connell et al., 2011), based on a remote camera survey in 2011 and 2012. We designed a standard sampling protocol developed for small to medium-sized felids (Trolle and Kery, 2003; Dillon and Kelly, 2008), including wildcats (Anile et al., 2010; Emre Can et al., 2011; Anile et al., 2012) and feral cats (Bengsen et al., 2011). Six sampling blocks were selected, each representing one of the main woodland formations of the Sierra Nevada (Fig. 1); the distribution of the six blocks was also affected by accessibility. Nine to twelve camera stations (just one camera per station) were set up in each sampling block (Table 1), 1–2 km apart (Fig. 1). The sampling period was 10 weeks for each block, with three sessions of two simultaneous blocks: spring 2011 (blocks 2 and 4), spring 2012 (blocks 3 and 6) and autumn 2012 (blocks 1 and 5; see Table 1 for further details). We used passive infrared-triggered cameras (DLC Covert – Covert Scouting Cameras, Lewisburg, KY, USA; and Leaf River IR-5–Leaf River Outdoor Products, Taylorsville, MS, USA). Live pigeons in cages were used as bait, following Gil-Sánchez et al. (2011); this procedure increases both the detection probability (see file S1 in the supplementary online Appendix) and the number of pictures per capture, hence improving the identification of individuals (Guilt et al., 2010; Garrote et al., 2012; Soto and Palomares, 2014). We also tried to systematically collect scats of wild-living cats for genetic identification of individuals, following a sampling protocol similar to that described by Anile et al. (2014); however, after an intensive sampling effort (12.8–20.1 km walked for each block; 92.03 accumulated km) by a well-trained person (J.M. Gil-Sánchez; see Gil-Sánchez, 1998; Gil-Sánchez et al., 1999a; Moleón and Gil-Sánchez, 2003; Gil-Sánchez et al., 2006), just three putative wildcat scats were found; thereafter, this method was dropped.

Since we knew that stray cats were fairly common within the villages of the Sierra Nevada, we designed a rapid survey to estimate their numbers at an order of magnitude level; this approach was sufficient to achieve our goal of determining the presence of a large population source of feral cats. Thereafter, the population size of the stray cats was determined by capture–recapture analysis based on the two capture samplings of Lincoln and Petersen (Williams et al., 2001), as well as by direct counting (Tennent and Downs, 2008; Goszczyński et al., 2009) in 2012 and 2013. First, we selected the village nearest to each one of the six camera sampling blocks which were located within the same valley as the main potential source of domestic cats (Fig. 1). Then, a walking survey of 1.1–3.2 km was designed to be carried out by following some streets in each village; after the first survey a second one was made following the same track (registered by GPS) after waiting half an hour. This short time delay improved the environmental homogeneity of both surveys. Most of the recaptured cats had moved away from the site of first capture, hence both surveys could be assumed to be sufficiently independent. During the walking surveys, each cat seen was photographed to enable individual identification (Tennent and Downs, 2008). Transects were walked in the morning during warm temperatures (>20 °C) and without rain to improve the detection rate (Goszczyński et al., 2009).

2.3. Data analysis

For analyses, only adult or sub-adult cats were taken into account to avoid seasonal effects. First, we morphologically identified each individual (wildcat, domestic cat or hybrid cat) by the coat patterns and the shape of the tail (Kitchener et al., 2005; Anile et al., 2012). A high concordance between genetic and pelage approaches for discriminating wildcats and hybrids has been confirmed, after careful examination of discriminatory characters from photographs (Ballesteros-Duperón et al., 2015). Wildcats were identified in two complementary ways: (i) We used photos of 12 pure wildcats belonging to the study population that were genetically identified by nuclear microsatellites (Oliveira et al., 2008b; Ballesteros-Duperón et al., 2015) as reference (Fig. 2). A captured

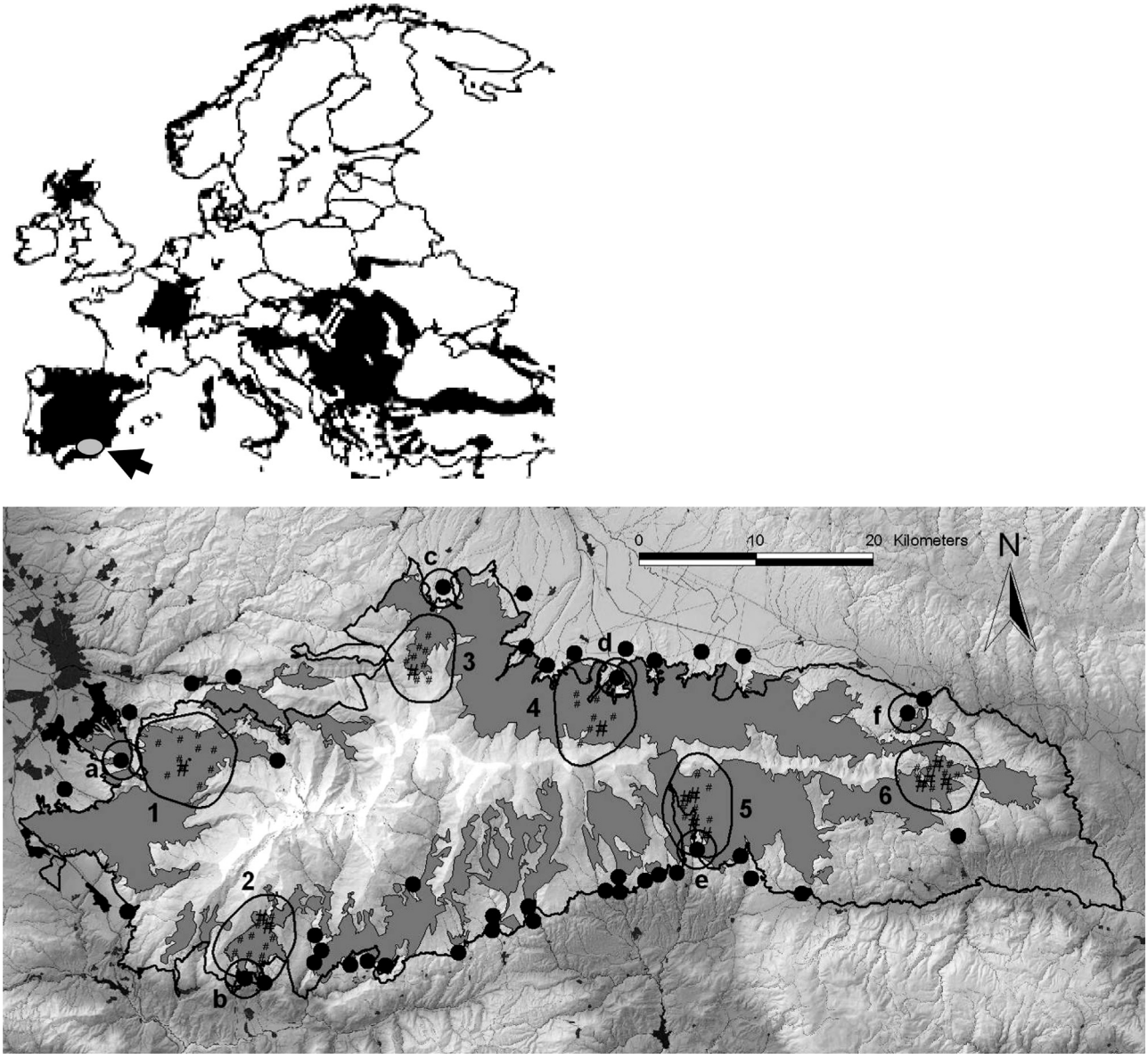

Fig. 1. Above: Location of the study area (arrow) with the distribution range of the European wildcat (map derived from The IUCN Red List of Threatened Species). Below: Sierra Nevada National Park limits with the locations of the six effective sampling areas of camera-trapping surveys (1–6) and walking surveys in six villages (a–f). Camera stations are represented by the symbol # (the large symbols indicating wildcat captures). Black polygons and dots are small and large villages, respectively; forests (wildcat habitat) are represented by the grey area.

Table 1
Results of the camera-trap surveys. A_{MMDM}, effective sampling area estimated from the mean maximum distances moved obtained from the data of the camera-trap samplings; A_{RT}, effective sampling by using data of three wildcats radio-tracked in the study population.

Block (Fig. 1)	# Camera stations	Dates	A_{RT} (km^2)	A_{MMDM} (km^2)	# Camera days	# Individual wildcats (# wildcat captures)	# Individual feral cats (# feral cat captures)
1	10	October–December 2012	39.97	57.51	686	1 (1)	0 (0)
2	12	April–June 2011	16.77	25.44	840	2 (3)	2 (3)
3	9	May–July 2012	12.71	19.74	544	1 (1)	0 (0)
4	10	May–July 2011	37.54	54.62	686	1 (1)	0 (0)
5	11	October– December 2012	34.47	52.23	763	3 (10)	0 (0)
6	12	April–June 2012	18.70	29.03	759	5 (37)	0 (0)
Total	64	–	160.16	238.57	4278	13 (53)	2 (3)

individual was assumed to be a wildcat when both the coat and tail were quite similar to the reference wildcats. (ii) We used the criterion of Kitchener et al. (2005) to detect any features present in the putative wildcats that might indicate some level of introgression and thus a hybrid status of the individual. However, rare cryptic hybrid individuals might have been possible (Devillard et al., 2014); therefore, the identifications should be taken with some caution. On the other hand, all cats living in villages were assumed to be

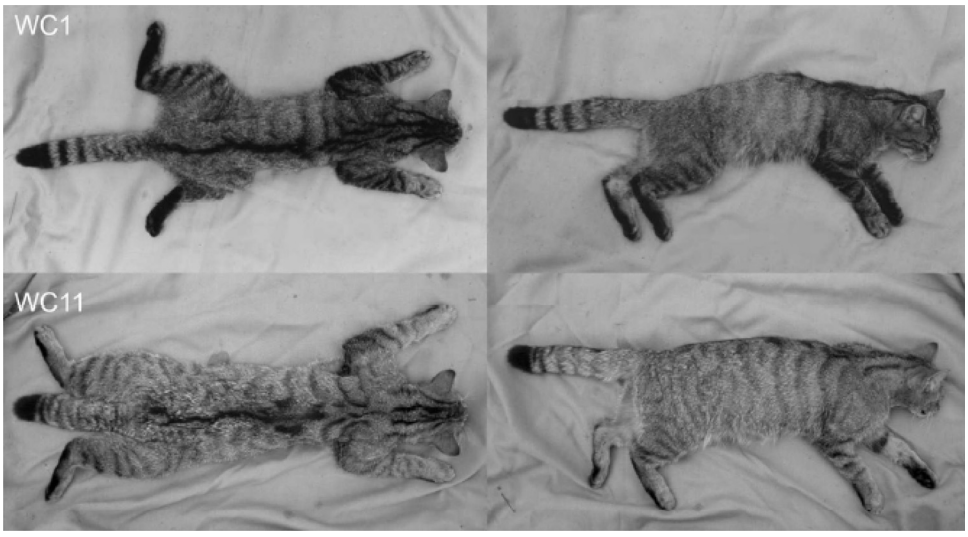

Fig. 2. Examples of two genetically pure wildcats of the study population. Both were live-captured for a radio-tracking study.

domestic cats, and all feral cats could unequivocally be identified as domestic cats (no traces of wildcat characters observed). Once the taxonomic status was established, each cat was individually identified following the protocol of Anile et al. (2012). Photographs of both sides were obtained for most of the wildcats, which allowed their correct identification (even for the two cats that where photographed only on the right side).

For wild-living cats we attempted to apply spatial capture–recapture (SCR) models for density calculations (Royle at al., 2014), following a protocol used for wildcats (Anile et al., 2014). However, sufficient recaptures to run models were obtained for only one block (# 6; Table 1); thereafter, we used a capture–recapture (CR) approach. First, a unique matrix with the capture history of each individual was generated from the data of the six sampling blocks and was treated as if they had all begun simultaneously (O'Connell et al., 2011), assuming that the population was stable over the camera-trapping period (April 2011–December 2012; Table 1); a period of seven days was used as sampling duration. The total number of cats ($\tilde{N}$) was estimated by the program CAPTURE (Rexstad and Burnham, 1991), after performing the closure test described by Stanley and Burnham (1999). To estimate the density (D), we calculated the effective sampling area A in two ways: (i) A_{MMDM}, using the mean maximum distances moved (MMDM), obtained from the data of the camera-trap samplings (see Anile et al., 2010, 2012 for details); and (ii) A_{RT}, using data of three wildcats of the study population radio-tracked some time ago (Gil-Sánchez et al., unpublished data). Wildcats were captured using double-entrance, electro-welded mesh box traps (2 m × 0.5 m × 0.5 m) baited with pigeons and fitted with VHF radio collars (Nylon Collars; Biotrack Ltd., Wareham, UK). The radio-tracking process consisted of obtaining one to two locations by triangulation every week, so that points could be assumed to be independent (Millspaugh and Marzluff, 2001). Following the method of Balme et al. (2007), for each sampling block, a buffer of 1878 m calculated from the mean home range radius (mean 95% min. convex polygon = 11,09 km^2) of the radio-tagged wildcats was applied on the minor convex polygon of the camera network. Non-forested areas inside the effective polygons were excluded for A calculations. The total population in the Sierra Nevada (N) was calculated by extrapolating D to the total area occupied by woodlands (80.177 ha). To study the accuracy of the CR ad hoc approach, densities were estimated from both CR (with A calculated as previously explained) and SCR models for the only block with sufficient captures for calculations (# 6, Fig. 1). We performed data analyses in the R statistical environment (version 3.1.1; R Development Core Team, 2014) by fitting the model SCR0 in BUGS (see file S2 in the supplementary online Appendix). Details of model SCR0 can be found in Royle et al. (2014). In this case, we did not exclude the non-forested areas for calculations since this was only used for a comparison of methods.

Domestic cats living in each village were estimated from the two captures using the Lincoln–Petersen method (Williams et al., 2001):

$$N = [(n_1 + 1)(n_2 + 1)]/m_2 + 1$$

$$Var(N) = [(n_1 + 1)(n_2 + 1)(n_1 - m_2)(n_2 - m_2)]/(m_2 + 1)^2(m_2 + 2)$$

$$95\%CI = N \pm 1.96\sqrt{Var(N)}$$

With n_1 being the number of cats detected during the first survey; n_2 the number of cats detected during the second survey; and m_2 the number of cats re-captured. $\tilde{N}$ was standardized as cats / km of streets, or KAI_N; then, the total population of each sampled village (N_1) was estimated as $N_1 = KAI_N$ x total length of streets. The density of cats (D) was calculated as $D = N_1$ / km^2 occupied by the sampled villages. Finally, since we assumed that the six villages (mean size = 0.13 km^2) sufficiently represented the rest of the village types (mean size = 0.14 km^2, $n = 54$ excluding only one large population of 11.13 km^2 at the western border, see Fig. 1), the total population (N_2) was calculated by extrapolating the mean D and its 95% CI ($n = 6$) to the total area occupied by the 55 villages located in the Sierra Nevada valleys (25.35 km^2).

To study the spatial segregation between wildcats and feral domestic cats, we used Cole's index (Cole, 1949) applied to the camera station data:

$$C = [(2AB/A + B)] \times 100$$

where A is the number of camera stations with wildcats, B is the number of camera stations with feral domestic cats, and AB is the number of camera stations with both wildcats and feral domestic cats. The index ranges between 0 (no coincidence at all) and 100 (total coincidence). We also analyzed whether wildcats showed any spatial segregation from villages (hence, from domestic cats) by using the Mann–Whitney U test to compare the distance to the nearest village of positive (known presences) and negative camera stations (pseudo-absences). Finally, we analyzed the relationship between the abundance of stray cats and the abundance of feral

cats within the same valley using the non-parametric Spearman correlation. The ArcView program (ESRI, Redlands, CA, USA) was used for spatial calculations.

3. Results

Putative wildcats were detected in all of the six blocks (1–4 positive camera stations per block, Fig. 1), whereas unequivocal domestic cats were found at only two camera stations in one block (Table 1). Thirteen wildcats were identified (Fig. 3); only one individual (BA-H1) bore a feature (alignment of tail bands) that might suggest some genetic introgression (Kitchener et al., 2005); however, this feature was also found in genetically pure local individuals (Fig. 2; Ballesteros-Duperón et al., 2015), and therefore all putative wildcats were assumed to be genetically pure. The closure test confirmed the assumption of a closed population ($\chi^2 = 5.63$; d.f. = 7; $P = 0.58$). The probability of capture was 0.23; the heterogeneity model (M(h)) was the best fitting model. Calculations were: $\tilde{N} = 15 \pm 3.06$ wildcats (95% IC = 14–30); $D_{RT} = 0.093 \pm 0.019$ wildcats/km^2 with total population = 74 (95% IC = 70–150); $D_{MMDM} = 0.062 \pm 0.012$ wildcats/km^2 (MMDM = 2681 m) with total population = 50 (95% IC = 47–101). For block #6, the closure test also confirmed a closed population ($\chi^2 = 2.63$; d.f. = 7; $P = 0.91$); the probability of capture was 0.38 and M(o) was the best model. Calculations were: $\tilde{N} = 5 \pm 0.21$ wildcats (95% IC = 5–5). The density of block #6 calculated by model SCR0 was 0.16 ± 0.05 wildcats/km^2 (see file S3 in the supplementary online Appendix for further details), density by CR_{RT} was 0.14 ± 0.006 wildcats/km^2 ($A_{RT} = 34.26$ km^2) and density by CR_{MMDM} was 0.09 ± 0.004 wildcats/km^2 ($A_{MMDM} = 53.19$ km^2). Only two domestic cats were registered by camera traps. Both individuals were located near inhabited country houses (560 m and 530 m) within the same block (Table 1). In the case of feral and domestic cats, insufficient data were gathered for CR calculations, and therefore only the minimum known density was calculated by # of captured individuals/A_{RT}, resulting in 0.014 individuals/km^2 (95% CI = ± 0.031; $n = 6$). This density suggested a minimum total population of 11 cats (95% CI = 0–36). Population estimations of stray cats ranged from 69.99 ± 32.85 to 188.17 ± 68.78 individuals per village (Table 2); the average D was 893.8 ± 309.01 cats/km^2 and N_2 (total estimated population for Sierra Nevada) was 22659.09 ± 7802.50 stray cats.

Cole's index for wildcats and feral cats was 0%. The distance to the nearest village was similar ($U = 326.0$; $Z = -0{,}092$; $P = 0.92$) for camera stations with wildcat captures (mean = 4.22 km) and camera stations without them (mean = 4.21 km). No relationship between stray cat and feral cat abundances within the same valley was found ($R_s = 0.39$; $P = 0.44$; $N = 6$).

4. Discussion

Our assumption that the population of wildcats was stable over the camera-trapping period (20 months) may bring a bias to the CR estimations (Foster and Harmsen, 2012); however, most of the sampling period was during 2012 and 49 out of the 53 wildcat captures were also made in 2012. Hence, it may be assumed that the effect of the long sampling time was not significant. In fact, intensive tracking in snow and scat surveys during 2012 over the two blocks sampled in 2011 showed a very low abundance of wildcats. The density calculated by model SCR0 for sampling block #6 was higher than the value given by CR_{MMDM}, but similar to that calculated by the radio-tracking-based CR_{RT}. Hence, the global population size predicted by CR_{RT} can be assumed to be more realistic. Anile et al. (2012, 2014) found similar densities of wildcats in Sicily from CR_{MMDM} and SCR estimations, but they obtained higher re-capture rates than we did, leading to more precise values for MMDM.The estimated wildcat density in the Sierra Nevada (0.093 ± 0.019 wildcats/km^2) was only half that of two camera-trap studies conducted in Sicily (Italy) (0.28 ± 0.1 wildcats/km^2; Anile et al., 2012) and Turkey (0.22 ± 0.06 wildcats/km^2; Emre Can et al., 2011). Densities calculated with other methods ranged between 0.1/km^2 and 0.5/km^2 for the European range of the species (Dimitrijevic, 1980; Okarma et al., 2002; Heltai et al., 2006; Kery et al., 2010). Thus, the abundance of wildcats in the Sierra Nevada can be assumed to be low. Once human-related causes of mortality (currently rare in the Sierra Nevada) have been excluded, this low density can be mainly explained by low food abundance: rabbits (*Oryctolagus cuniculus*), a key prey for wildcats in their Mediterranean range (Lozano et al., 2006), are very scarce in the Sierra Nevada (Moleón and Gil-Sánchez, 2003); in fact they were absent in three of the six sampled blocks. A similar hypothesis has been proposed to partially explain the low apparent abundance of wildcats in the Doñana National Park in south–west Spain (Soto and Palomares, 2014).

The abundance of feral cats was two orders of magnitude lower than that registered in non-Mediterranean areas of Europe. For example, feral cat densities of up to 2.5–3.3 individuals/km^2 have been reported in southern Sweden (Liberg, 1980), and a relative abundance of 4.9 cats per 100 camera-trapping days was recorded in the Jura Mountains of Switzerland (Eichholzer, 2010), whereas for the Sierra Nevada the value was 0.046. For other Mediterranean protected areas, 0.032 and 0.065 feral cats per 100 camera-trapping days were obtained in Doñana National Park (Soto and Palomares, 2014) and Serra da Malcata, Portugal (Sarmento et al., 2009), respectively. Moreover, Ferreira (2010) failed to detect cats in areas far from human settlements in southern Portugal (trapping effort more than 4000 box-trap nights). Therefore, it seems that the habitats of the temperate moist European areas hold a greater abundance of wild-living cats than do Mediterranean landscapes.

The direct counting of domestic cats in the villages may have been biased, since some of the stray cats could have been inside houses; hence, the assumption of homogeneity of capture probability in the statistical model (Williams et al., 2001) would be incorrect; in this case our approach may underestimate the real population. On the other hand, stray cats may use areas surrounding village limits. Therefore, extrapolating density without accounting for this difference in spatial distribution may lead to some overestimations (which could compensate for the prior potential underestimation). Regardless, even considering the limitations of our Lincoln-Petersen method approach, it is clear that the villages of the Sierra Nevada hold important populations of domestic cats, with numbers two orders of magnitude higher than those of the wildcat population. According to Liberg et al. (2000), populations with more than 100 stray cats/km^2 are found where optimal food scenarios exist, as is the case in the Sierra Nevada. Abundance values for stray domestic cats of even more than 2350 cats/km^2 have been reported elsewhere (Sunquist and Sunquist, 2002).

The fact that the distance to the nearest village was not different between positive and negative camera stations suggests that wildcats may use areas close to these human settlements. However, the fact that wildcats inhabit forests 0.52 km (mean value) from villages, along with their low density and, likely, shy behavior appears to discourage any mating between stray cat males and wildcat females, a sexual behavior that would produce F1 hybrids in the wild. On the other hand, in spite of the widespread presence of potential sources of feral cats (villages), the local abundance of this type of cats was extremely low. The null value of Cole's index could thus be related to the scarcity of feral cats rather than to the low capture probability of wildcats; indeed, the block where feral cats were detected was the only one with inhabited country houses, and hence those two detected individuals may instead have been

Fig. 3. Selection of camera-trap pictures of the 13 wildcats detected during the field survey.

roaming house cats. Nonetheless, there was obviously some overlap at a landscape level and therefore some risk of cross-mating. Our data support the idea that the scarcity of successful incursions of domestic cats into the forests of the Sierra Nevada is the major reason for the pure genetic status of the wildcat population in this area. Since human persecution may be excluded, given the protected status of the study area, four complementary explanations may be proposed:

(1) Food limitation. A field study in southern Portugal, in a well-preserved natural area with small scattered local farms, showed

Table 2
Results of the walking surveys of domestic cats within villages. n_1, number of cats detected during the first survey; n_2, number of cats detected during the second survey; N_1, estimated total population of each sampled village; D, estimated density of cats.

Village (Fig. 1)	n_1	n_2	m_2	Walked km	Total km streets	Total km^2	$N_1 \pm 95\%$ CI	$D \pm 95\%$ CI
a	10	6	2	2.6	5.27	0.1847	69.99 ± 32.85	378.93 ± 177.85
b	13	12	7	1.65	5.58	0.0737	75.37 ± 20.39	1022.65 ± 276.66
c	5	2	1	1.11	5.16	0.0683	46.49 ± 22.31	680.67 ± 326.64
d	13	14	5	3.23	16.37	0.2162	184.3 ± 76.93	852.54 ± 355.82
e	8	12	4	1.18	6.29	0.0831	127.93 ± 52.18	1539.47 ± 627.91
f	15	9	5	2.3	16.03	0.2117	188.17 ± 68.78	888.85 ± 324.89

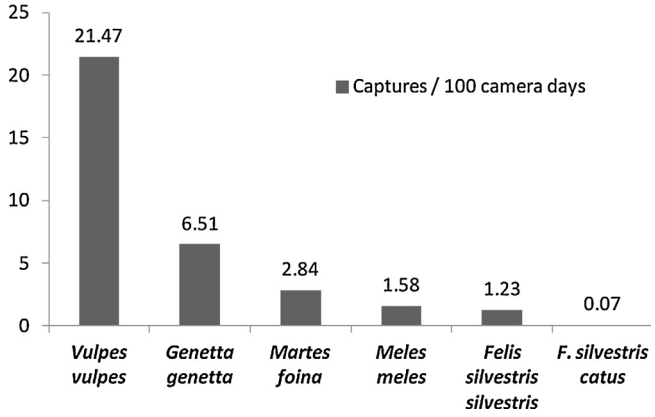

Fig. 4. Relative abundance of carnivores in the Sierra Nevada derived from camera-trapping surveys from the present study.

that the presence, abundance and space use of farm cats depended heavily on human settlements, with their diet demonstrating their dependence on people (Ferreira, 2010). This may be similar to the ecological scenario in the Sierra Nevada. Another important factor is the low abundance of rodents (especially voles) in Mediterranean areas (Moreno and Barbosa, 1992).

(2) Intra-specific competition. Felids are carnivores with intrasexual spatial exclusion (Sunquist and Sunquist, 2002) through active territorial displays including direct aggression or even intraspecific kills (Mattissona et al., 2013). Since wildcats are larger than domestic cats (Sunquist and Sunquist, 2002), it can be hypothesized that, within a healthy population of wildcats, domestic cats would face difficulties in gaining and holding a territory. In fact, mixed populations of wildcats and feral cats have been reported mostly in areas where wildcats are declining from other causes (Lozano and Malo, 2012).

(3) Intra-guild competition. This is a key interaction of carnivore communities (e.g., Fuller and Keith, 1981; Creel and Creel, 1996; Palomares et al., 1996). In our study area, exploitation competition may occur with red fox, stone marten and genet, since these three carnivore species also prey mainly upon rodents (Gil-Sánchez, 1998; Padial et al., 2002) and are far more abundant than feral cats (Fig. 4). In a field study on interactions between red foxes and feral cats in Australia (Molsher, 1999), three radio-collared cats were killed by foxes and aggression was observed toward cats (interference competition); in fact, inter-specific competition was considered to be the most likely mechanism limiting feral cats (Molsher, 1999).

(4) Predation by large raptors. The Sierra Nevada holds substantial populations of golden eagle (*Aquila chrysaetos*) and eagle owl (*Bubo bubo*) (Gil-Sánchez et al., 1999b). Both raptors prey upon cats (Mikkola, 1983; Watson, 1997) and the usually non-cryptic phenotypes of the domestic cat make them easy to locate. These raptors (mainly the eagle owl) appear to be common predators of domestic cats on farms in south-eastern Spain, according to local people that we interviewed.

In summary, the potential interplay among environmental and demographic variables may play a key role in the genetic health of the wildcat population, working at two basic levels: (i) hampering the demographic flux of domestic cats into the forests and (ii) limiting crossbreeding opportunities through spatial segregation. Most of the four potential barriers for domestic cats mentioned above are very typical not only of the Sierra Nevada, but also throughout the Mediterranean mountains of the Iberian Peninsula: namely, low vole abundance (with only one widespread species, *Microtus duodecimcostatus*; Palomo and Gisbert, 2002), a strong presence of meso-carnivores (up to eight species; Palomo and Gisbert, 2002), and well-preserved populations of large raptors (up to four species, including *Aquila adalberti* and *Aquila fasciata*; e.g., 1553–1769 pairs of golden eagle were estimated to live in Spain; Del Moral, 2009). The observed divergences regarding the hybridization problem across the regions of Europe could be explained within this context, although further research is necessary to understand the complex relationships between the demography of cats and their environment.

5. Conclusions

Active control of feral cats has been proposed as a key conservation strategy for the European wildcat (Daniels and Corbett, 2003). However, this is a costly measure with logistical and ethical limitations and should therefore only be implemented on a large scale in areas where hybridization has been detected (Lozano and Malo, 2012). Our study highlights the large differences that exist in ecological features and conservation scenarios throughout the distribution range of the wildcat and shows that, at least in some areas of the Mediterranean mountains of the Iberian Peninsula, such measures are not necessary.

On the other hand, our work also shows some important limitations of noninvasive methods (camera-trapping but also DNA sampling from scats) when low-density populations of wildcats are studied. These populations may be widespread throughout the distribution range of the species, as in the case of southern Spain, where there remain important areas for wildcats (Palomo and Gisbert, 2002; Lozano and Malo, 2012). It is important to emphasize that in the present study the camera-trapping survey offered the best results compared with scat surveys, a method that resulted in four false negatives out of six cases. Hence, the detailed examination of discriminatory characters from pictures (Ballesteros-Duperón et al., 2015) taken by appropriate camera-trapping surveys could become a key approach in the case of low population densities. In order to improve the capture rates in further surveys of wildcats with camera traps we recommend not only to increase the size of the sampling areas and the number of camera stations, but also to extend the sampling period and to use lures (Kery et al., 2010; Monterroso et al., 2011; Soto and Palomares, 2014).

Acknowledgements

We thank Rogelio López, Jesús Bautista, María D. Martínez, Carolina Porto, Carmen Jiménez and Antonio Martín for their valuable help with field work. Javier Sánchez, Ignacio Henares, Blanca Ramos and Ignacio Maldonado provided technical and administrative support. José Jiménez kindly helped with the SCR analysis of data. The research was funded by the Consejería de Medio Ambiente y Ordenación del Territorio through the European Union (FEDER Project) and is part of the Global Change Observatory of Sierra Nevada. Paulo Celio Alves helped improve early versions of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.zool.2015.08.001.

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