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Wildcat population density on the Etna volcano, Italy: a comparison of density estimation methods

S. Anile[1], B. Ragni[2], E. Randi[3], F. Mattucci[3] & F. Rovero[4]

1 Dipartimento di Biologia Animale 'Marcello La Greca', Università di Catania, Catania, Italy
2 Dipartimento di Biologia Cellulare ed Ambientale, Università degli Studi di Perugia, Perugia, Italy
3 Laboratorio di genetica, Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Ozzano dell'Emilia, BO, Italy
4 Sezione di Biodiversità Tropicale, MUSE – Museo delle Scienze, Trento, Italy

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Correspondence
Stefano Anile, Dipartimento di Biologia Animale 'Marcello La Greca', Università di Catania, Via Androne 81, Catania 95124, Italy.
Email: stefanoanile@yahoo.it

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Abstract

The European wildcat is an elusive felid that is declining across its range. Sicily hosts a distinctive insular wildcat population, the conservation of which requires much better ecological knowledge than is currently available, particularly population density. We simultaneously used two noninvasive methods (camera-trapping and scat-collection) to estimate the population density of wildcats on the Etna volcano. We conducted genetic analyses to identify individuals and to detect potential hybridization with the domestic cat. We analyzed individual capture-histories from camera-trapping and scat-collection using the spatially explicit capture-recapture (SECR) model. Furthermore, we applied the random encounter model (REM), which does not require individual identification, to the camera-trapping data. We identified 14 wildcats from 70 photographic detections (6.48 detections/100 trap-days) obtained from 1080 camera-trapping days over 4 months, and we estimated to have identified all the individuals living in the study area (10.9 km^{-2}). On the contrary, we identified 10 wildcats from 14 out of 39 scats collected from 391 km of transects walked. The estimated densities (individuals $km^{-2} \pm$ SE) were 0.32 ± 0.1 (SECR camera-trapping), 1.36 ± 0.73 (SECR scat-collection) and 0.39 ± 0.03 (REM). The population density estimates obtained from SECR camera-trapping and REM overlapped, although we recommend care when applying the latter. The SECR scat-collection gave the highest population density (and less precise) estimates because of the low number of capture and recaptures; however, the population size estimated with this method matched the number of individuals photographed. The population density of the wildcat in Etna falls in the medium-high range of those reported in literature, highlighting the role of this ecosystem for the long-term conservation of the wildcat in Sicily. Camera-trapping is confirmed as a useful tool to assess the wildcat population density and, in this case, was complemented by the genetic analysis that confirmed individual identity.

Introduction

The European wildcat (*Felis silvestris silvestris* Schreber, 1777) is widespread throughout Europe in fragmented and scattered populations occupying different habitats ranging from scrub-pasture lands (Lozano *et al*., 2003; Monterroso *et al*., 2009; Lozano, 2010) to forest patches intermixed with fields (Klar *et al*., 2008, 2012). Limited human disturbance is a fundamental requirement for the persistence of viable wildcat populations (Klar *et al*., 2008; Monterroso *et al*., 2009; Piñeiro & Barja, 2012; Piñeiro *et al*., 2012). Currently, the European wildcat is classified as Least Concern by the IUCN; however, the population is decreasing throughout its range (Driscoll & Nowell, 2010) because of the loss of suitable habitat (Klar, Herrmann & Kramer-Schadt, 2009; Klar *et al*., 2012), mortality on roads (Krone *et al*., 2008), overgrazing by large game species (Lozano *et al*., 2007) and hybridization with the domestic cat (*Felis silvestris catus*; Oliveira *et al*., 2008). Hybridization is widely documented across the European distribution of wildcats, with highest levels for Scotland (Daniels *et al*., 2001; Kilshaw & Macdonald, 2011) and Hungary (Lecis *et al*., 2006), and low hybridization levels in France (Germain, Benhamou & Poulle, 2008), Spain, Portugal (Oliveira *et al*., 2008) and Germany [although with contrasting results (Eckert *et al*., 2009; Hertwig *et al*., 2009)].

The genetic structure of the European wildcat in Italy has been recently described by Mattucci *et al*., (2013) who showed that the Sicilian population, the only Mediterranean insular

population that is not the result of human introduction (Kitchener & Rees, 2009), represents a distinctive wildcat conservation unit (Mattucci *et al.*, 2013). Conservation measures for the Sicilian wildcat are needed because not only is the overall population size declining, but the isolated nature of the island does not allow recruitment from neighboring populations. However, very few studies have been conducted on this unique wildcat population (Anile, Amico & Ragni, 2012).

Wildcats typically occur at low population density, and in Mediterranean ecosystems, they live in mosaic landscapes of mixed shrubby vegetation, open areas and patches of forest, making wildcat population density estimation a challenging task (Kery *et al.*, 2011). Thus far, only a few studies have tried to assess wildcat population density using noninvasive methods like camera-trapping (Can, Kandemir & Togan, 2009; Kilshaw & Macdonald, 2011) or hair-trapping (Kery *et al.*, 2011). The development of spatially explicit capture-recapture (thereafter, SECR) models applied to capture-mark-recapture data has overcome the problem of how to calculate the study area to which the estimated population density refers, thereby reducing the major source of bias and allowing flexibility in the study design (Efford, Dawson & Borchers, 2009).

Another recent development for estimating population density from camera-trapping is the random encounter model (hereafter, REM) proposed by Rowcliffe *et al.* (2008) in which the population density is derived directly from the trapping rate; therefore, individual identification is not necessary. The REM is based on gas collision theory (with animals as 'random' molecules and cameras as fixed molecules), and it has been tested in wild conditions on only a few occasions (Rovero & Marshall, 2009; Manzo *et al.*, 2012). We applied the REM to our data because of the following considerations. First, wildcats usually live at low population density (Kery *et al.*, 2011); therefore, gaining enough recapture records to run density models can be impossible in some areas (Kilshaw & Macdonald, 2011). Second, capture-recapture studies usually require two camera-traps per station to achieve better individual identification (Kilshaw & Macdonald, 2011). Therefore, using only one camera per station, as required by the REM, doubles the potential sampling area. Finally, thus far, only one study has tested the REM against an independent population density estimate in the wild (Rovero & Marshall, 2009), and hence, we also aimed to provide an additional test of this model in the field.

Standardized protocols are required to produce reliable estimates for threatened taxa, such as the European wildcat, which in turn can inform conservation plans (Mattucci *et al.*, 2013). Additionally, the integration of different sampling techniques could strengthen the overall study outcome and provide useful insights to improve future research efforts (Janecka *et al.*, 2011). Therefore, we aimed to estimate the wildcat population density on Mount Etna (Sicily) using two independent and noninvasive survey methods, camera-trapping and scat-collection, followed by SECR analyses. Furthermore, we also applied the REM to our camera-trapping data to compare its population density estimate with those of the SECR models.

Materials and methods

Study area

The study area (10.1 km^{-2}) was located on the south-west side of Mount Etna, Sicily and ranged from 900 to 2.000 m a.s.l., corresponding to the southernmost location of the wildcat distribution in Italy (Nowell & Jackson, 1996) (Fig. 1). The Etna Regional Park (59 km^{-2}) may play an important role in wildcat conservation for this island connecting the western wildcat populations with the eastern one (Anile *et al.*, 2012). The volcanic activity has produced a landscape characterized by recent large lava flows and inactive secondary cones of different ages, intermixed with areas dominated by trees (*Pinus laricio*, *Quercus pubescens*, *Quercus ilex*, *Castanea sativa*, *Populus tremulus* and *Genista etniensis*), and therefore, the resulting habitat consists of large woodland patches intermingled with open fields. Potential wildcat refuges are widely available in form of the cavities, characteristic of the volcanic soil, which also represent (due to water condensation on the cavity's walls) the only available water in summer time. Human activity is low, with vehicle access subjected to permission and restricted to nonpaved roads used for the management of woodland, sheep farming and tourist trekking. The climate is typically Mediterranean, but snow cover is common in winter. Rainfall is concentrated during autumn and winter season with a yearly mean of 1000–1400 mm.

Data collection

We used 18 digital camera traps [DFV® equipped with Sony® DSC-W55 (Fototrappolaggio, Forlì, Italy)]. Each camera trap was accommodated in an iron box, locked with a padlock and then tied to a tree (at 50 ± 10 cm from the ground) with a chain. We set camera traps with a delay time of 10 min between successive bursts ($n = 3$) of photos. Camera-trapping survey consisted of two trapping lines that were run for two consecutive periods (from 14 May to 11 September 2010, i.e. 120 days): each trap line (running for 60 days) included nine camera-trapping stations, each consisting of a pair of camera traps facing each other to obtain photographs of both flanks of a wildcat. We set the camera stations on paths that wildcats often travel along with variable widths (220 ± 80 cm). We angled cameras slightly downwards (15° ± 3°) in order to obtain a better view of the wildcat's *norma dorsalis* [part of the coat that is useful for identifying individuals (Ragni & Possenti, 1996; Kilshaw *in litteris*)]. This orientation also constrained the detection zone of camera traps to the width of the path. The interstation distance was 1351 ± 790 m so the whole study area (Fig. 1) was covered without leaving gaps large enough to include the smallest known home range for wildcat in Mediterranean habitat (1.81 and 1.70 km^{-2}; Sarmento *et al.*, 2006; Monterroso *et al.*, 2009); hence, we assumed all wildcats living in the study area had a probability >0 of being photographed. We did not use lures or baits to avoid potential bias because of differential responses of wildcats to attractants (Jackson *et al.*, 2005).

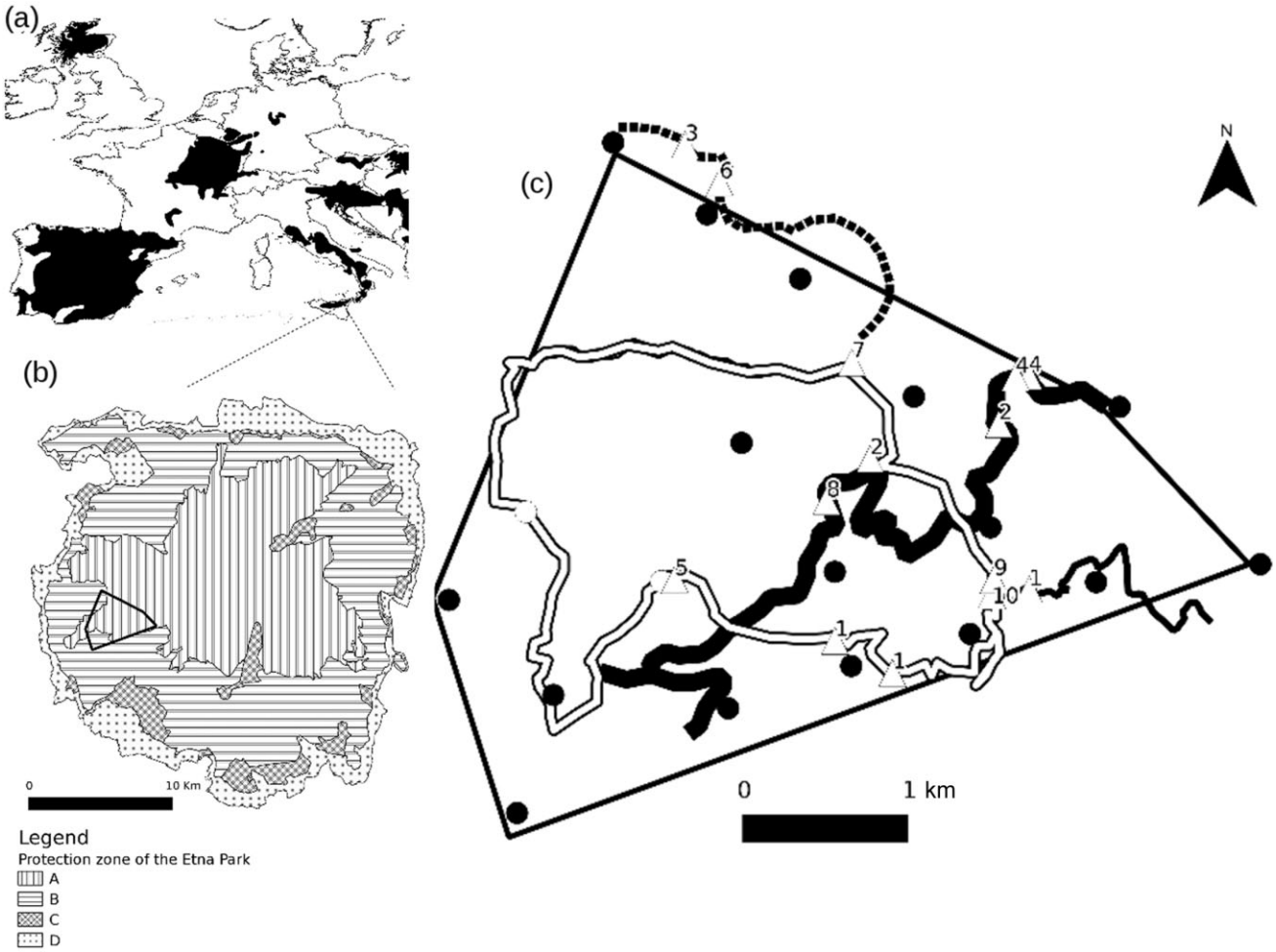

Figure 1 (a) Approximate wildcat distribution ranges for Western Europe. The low inset (b) shows the borders of the different protection zones in the Etna Regional Park in Sicily where we conducted the present study (horizontal lines = zone A, 'integral reserve' subjected to more constraints in order to ensure maximum protection of plants and animal species; vertical lines = zone B, 'general reserve' in which educational activities and excursions are permitted; square area = zone C, 'protection area' located close to population centers where eco-friendly activities are encouraged; dotted area = zone D, 'pre-park area' where landscape is dominated by medium-size traditional agricultural plantation mixed with the forest, and all the activities allowed have to comply with the general purpose of the park. The right inset (c) shows the location of camera stations. Filled points are the camera-stations positive for wildcat, while open points are the negative ones. The 10 individuals identified from the genetic analysis are represented by triangles. The minimum convex polygon encompassing the camera-stations is 10.9 km^{-2}.

Data analysis

Camera-trapping

We assessed the taxonomic status (domestic/wildcat/hybrid) of photographed cats using the system of coat coloration and markings proposed by Ragni & Possenti (1996). This morphological classification has proven to be highly congruent with the genetic results of several published studies (Randi *et al.*, 2001; Pierpaoli *et al.*, 2003; Lecis *et al.*, 2006; Oliveira *et al.*, 2008; Mattucci *et al.*, 2013). We identified the individuals using the following morphological criteria: number, shape, dimension and position of stripes, bands and spots on the trunk and limbs; number and shape of the rings on the tail; and dimension of black tail tip and unequivocal body signs such as scars on the face, lips and ears (Fig. 2). Additionally, in some cases, we were able to sex the wildcats according to the presence of external genitalia or if accompanied by kittens. In order to be accurate and conservative, two of us (S. A and B. R.) independently examined all the photographs, and we included only concordant individual identifications in the capture history. We constructed the spatial-history capture matrix for the camera-trapping data, whereby we associated each capture of the *i*-individual with the respective coordinates of the camera station and *i*-occasion, which consisted of 6 days; hence, there were 20 camera-trapping occasions.

Figure 2 Examples of wildcat morphological characteristics evaluated during the photographic comparison in order to perform individual identification of wildcats in the Etna Regional Park, Italy.

We performed data analyses in the R statistical environment (version 3.0.2, R Development Core Team, 2012) using the package *secr* (Efford, 2011) to calculate population density estimation under a SECR framework. Specifically, *secr* uses two distinct submodels within its workflow to compute the density (D): the first one simulates an animal's distribution from the capture history giving the individual's activity centre as its output, while the second simulates the capture process on the basis of the radial distance between the estimated centre of activity and the traps (Efford *et al.*, 2009).

We set trap detector type for the camera-trapping analysis as 'proximity' (allowing for multiple detections of the same individual within the same occasion). We used the buffer function in *secr* to obtain an estimate for the buffer value to use when running the analyses on both datasets. We set the parameters for *secr* analyses as follows to allow a comparison between the results of the two independents datasets:

- distribution = Poisson; a homogeneous distribution of home range centre in the study area seemed a reasonable hypothesis because of the high trap success across stations and small trapping area (Borchers & Efford, 2008);
- detection function = half-normal; we uniformly set the cameras on well-used paths instead of random or cluster locations; hence, we assumed that capture probabilities decreases linearly with the distance of camera station to the individual's home range centre (Efford, Borchers & Byrom, 2009).

Additionally, we applied the equation 4 $D = \frac{y}{t}\frac{\pi}{vr(2+\theta)}$ of Rowcliffe *et al.* (2008) to our camera- trapping data to estimate the wildcat population density (D) directly from the camera-trapping rate ($\frac{y}{t}$: y = # detections; t = unit time) that was calculated as follows. Given that all parameters except detections (y) in the equation were constant, we used detections (y) to estimate mean population density and 95% confidence intervals using a bootstrapping approach. First, we considered the wildcat detections from a single camera per station. Then, for each of this two datasets (one from the left camera and one from the right camera), we randomly sampled 18 values (corresponding to the 18 camera stations) for 10 000 times. Lastly, using the average of the means from these two datasets, we calculated the population density (D) along with the 95% confidence intervals. To quantify the variable v (animal speed/day-range) required by the REM, we used the average day-range (2.26 km) from available radio-tracking data for Mediterranean habitat (Sarmento *et al.*, 2006; Monterroso *et al.*, 2009). We measured the detection zone parameters of cameras, distance r and arc θ, with a series of trials ($n = 30$) in which the camera was approached, at varying speeds, by a leashed domestic cat (Manzo *et al.*, 2012). We induced the cat to move perpendicularly toward the camera at the distances of 1.4, 2.2 and 3 m (minimum, average and maximum value of the distance between camera traps in the same station, respectively) for 10 trials for each measurement.

Genetic analysis

We collected scat samples in the same area where we set cameras by walking four transects weekly (5.5 ± 2.1 km) and repeated for 17 consecutive occasions (total of 391 km walked) (Fig. 1). We walked transects mainly just after sunrise to minimize the exposure of those scats deposited during that night to sunlight and high-temperatures that could increase the likelihood of DNA degradation. In addition, we calculated the scat-deposition rate (n/km) for the fresh scats (excluding those collected in the first occasion as these could potentially biased the value) to provide a baseline reference for future surveys.

We identified wildcat scats following the criteria outlined in Lozano *et al.* (2003). We only analyzed fresh samples, i.e. those with a shiny surface, soft consistency and characteristic smell, to increase the chances of successful individual genotyping. We collected samples in 95% ethanol and stored them at −20°C less than 6 h after collection in the field. We automatically extracted total DNA using a MULTIPROBE II^{EX} Robotic Liquid Handling System (Perkin Elmer, Waltham, MA, USA) and the QIAGEN DNeasy tissue extraction kits (Qiagen, Inc., Hilden, Germany). We genotyped individuals at nine unlinked autosomal cat microsatellites (Menotti-Raymond & O'Brien, 1995; Menotti-Raymond *et al.*, 1999) selected because they have been reliably used in previous wildcat studies (Randi *et al.*, 2001; Pierpaoli *et al.*, 2003). We extracted and amplified scat samples in dedicated

rooms under a sterile laminar flood hood. We performed polymerase chain reactions (PCRs) following a multiple-tube approach designed for low-quality DNA samples (Taberlet *et al.*, 1996; Randi *et al.*, 2001). We always used negative (no DNA in PCR) and positive (samples with known genotypes) controls. We analyzed the amplicons in an ABI 3130xl sequencer with the software GENEMAPPER 4.0 (Applied Biosystems, Foster City, CA, USA). We assessed the quality of scat DNA by replicating independently four to eight amplifications per locus. After the first four replicates, we discarded samples showing ≤50% positive PCRs (i.e. PCRs producing the expected amplicons; screening step). We performed a reliability analysis (RELIOTYPE; Miller, Joyce & Waits, 2002) on samples showing >50% PCR+, and we additionally replicated four times unreliable loci (at threshold $R < 0.95$). We only definitively accepted genotypes with $R > 0.95$. We used GIMLET .3.3 (Valière, 2002) to compute the rate of allelic dropout (ADO), false alleles (FAs; Broquet, Menard & Petit, 2007) and to reconstruct the consensus genotypes. We accepted heterozygotes only if we saw the two alleles at least in two replicates, while homozygotes only if we saw the allele in at least four replicates. We used GENALEX 6.4 (Peakall & Smouse, 2002) to match the consensus genotypes among them and with a European wildcat database ($n = 202$).

We used the software STRUCTURE 2.3.1 (Falush, Stephens & Pritchard, 2003) to assign the genotypes to their population of origin, independent of any prior nongenetic information, to ascertain the taxonomic status of sampled individuals. The reference European wildcat samples included 202 genotypes collected from the entire species' distribution range in Italy, Sicily included. The reference domestic cats included 77 randomly bred cats collected within the wildcat range or in local catteries (Mattucci *et al.*, 2013). We replicated each run of STRUCTURE five times, with 10^4 burn-in followed by 10^5 simulations, with or without the 'admixture' model, with the correlated ('*F*') or the independent ('*I*') allele frequencies models. We identified the optimal number of populations *K* running the software with $K = 1$–10 and using the ΔK statistics implemented in STRUCTURE HARVESTER 0.6.7 (Evanno, Regnaut & Goudet, 2005). Following previous studies (Oliveira *et al.*, 2008; Randi, 2008), we assigned the unknown individual genotypes to one cluster (wild or domestic) if the proportion of membership was $qi \geq 0.80$ or to both clusters if the proportion of membership was $qi < 0.90$ (admixed individuals). We compared STRUCTURE results with those obtained using the frequency-based assignment test in GENALEX (Paetkau *et al.*, 1995).

We constructed a spatial-history capture matrix for the scat-collection datasets, whereby we associated each capture of the *i*-individual with the respective coordinates of the scat deposition place and *i*-occasion, which consisted of 7 days; hence, there were 17 scat-collection occasions. Following the same procedures used for the camera-trapping, we estimated wildcat population density using *secr* models. However, we set trap detector type for the scat-collection analysis as 'transect-independent' (allowing for detections of the same individual on different transects).

All results are presented as mean ± SE (unless explicitly stated). Population density estimations (*D*) are expressed as individuals km^{-2}.

Results

Camera traps accumulated 1080 trap-days and produced 70 wildcat detections from 16 out of 18 stations, yielding a trapping rate of 6.48 (# detections/100 trap-days). We identified 14 wildcats (excluding two kittens) from 59 detections (we discarded 11 wildcat detections because individual identification was not possible). Individual wildcats were photographed on average 4.2 ± 3.4 times with individual captures per station being 4.2 ± 2.8, and wildcats without recaptures ($n = 5$) were photographed only at the outermost camera stations. The number of individuals detected stabilized after the seventh occasion.

We collected a total of 129 wildcat scats, of which we considered 39 as fresh and therefore suitable for DNA extraction. Scat-deposition rate resulted in one fresh scat in 14.7 km. We discarded 24 samples (62%) that showed PCR+ ≤0.50 during the screening step of the multitube protocol. We amplified the other 15 samples (38%) that showed PCR+ >0.50 a further four times. Finally, we discarded only one sample (SA109), while we accepted the other 14 (36%) that reached the threshold $R > 0.95$ (Supporting Information Appendix S1). The genotyped scats showed ADO = 0.25 ± 0.05 and FA = 0.01 ± 0.01, and had low match probabilities MP (calculated as genotype probability *x* population size) = 1.2×10^{-3} and MPsibs (as estimated with PIDsibs) = 1.8×10^{-1}. The majority (85%) of the scats positive for the DNA extraction was collected within the first and the last two sampling occasions.

We identified 10 distinct individuals using the multilocus match (Supporting Information Appendix S1); we sampled one individual three times, two individuals two times and the others once. We obtained the highest values of ΔK in STRUCTURE with $K = 2$ and average $\mathrm{Ln}P(D) = -9170.28$ [$K = 1$ had $\mathrm{Ln}P(D) = -10077$, and $K > 2$ had $\mathrm{Ln}P(D) \leq 8656.77$]. At $K = 2$, we assigned all reference domestic cats (average $Q_D = 0.978$; individual *q*d ranging from 0.833 to 0.998) and European wildcats ($Q_W = 0.984$ and *q*w = 0.867–0.997) to two distinct clusters either using the '*I*' or '*F*' allele frequency model. We assigned the 10 genotypes identified in this study to the wildcat clusters with $Q_W = 0.976$ and individual *q*w ranging from 0.959 to 0.996 (Supporting Information Appendix S1 and Fig. 3). The frequency-based population assignment test in GENEALEX confirmed STRUCTURE results, assigning all baseline domestic and European wildcats to their own clusters, and the 10 new samples to the wildcat clusters with 100% probability (Supporting Information Appendix S1 and Fig. 4).

The population density estimates based on the SECR model for camera trapping dataset was D = 0.32 ± 0.10, with an overall efficiency of detection {i.e. detection probability, per sampling occasion, when the hypothetical activity centre of an animal coincides spatially with a camera trap; [g(0)]} of 0.14 ± 0.03, and a scaling parameter of the distance function (σ) of 1000 ± 1376 m. The mean trapping rates for the

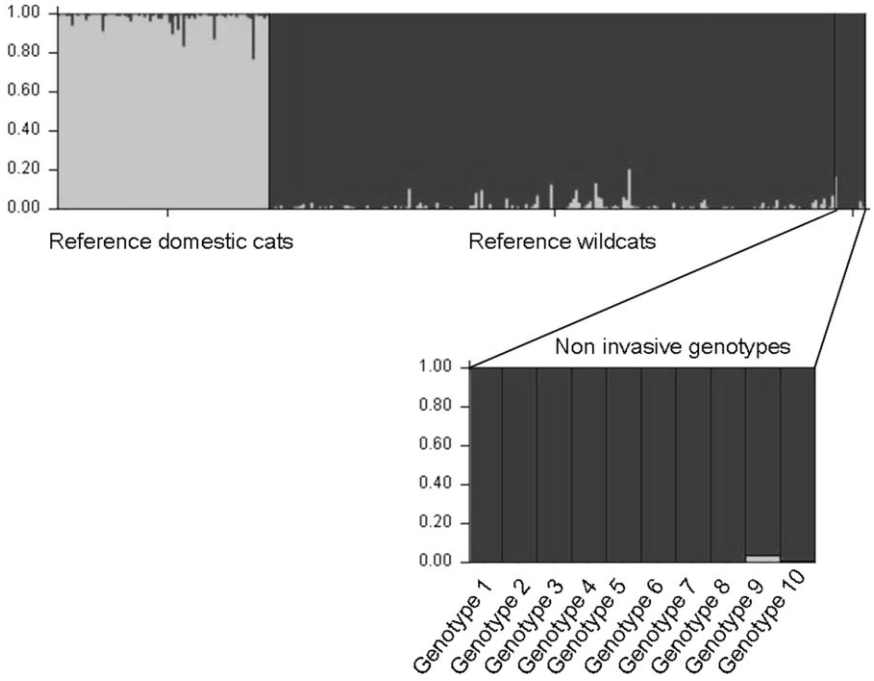

Figure 3 Plot of individual membership proportion to a domestic (gray) and wildcat cluster (black) obtained in software STRUCTURE (with the '*F*' or '*I*' models and choosing $K = 2$) in reference domestic and European wildcats and scat genotypes. Each cat genotype is represented by a vertical bar split in K colored sections, according to their membership proportion in the K genetic clusters.

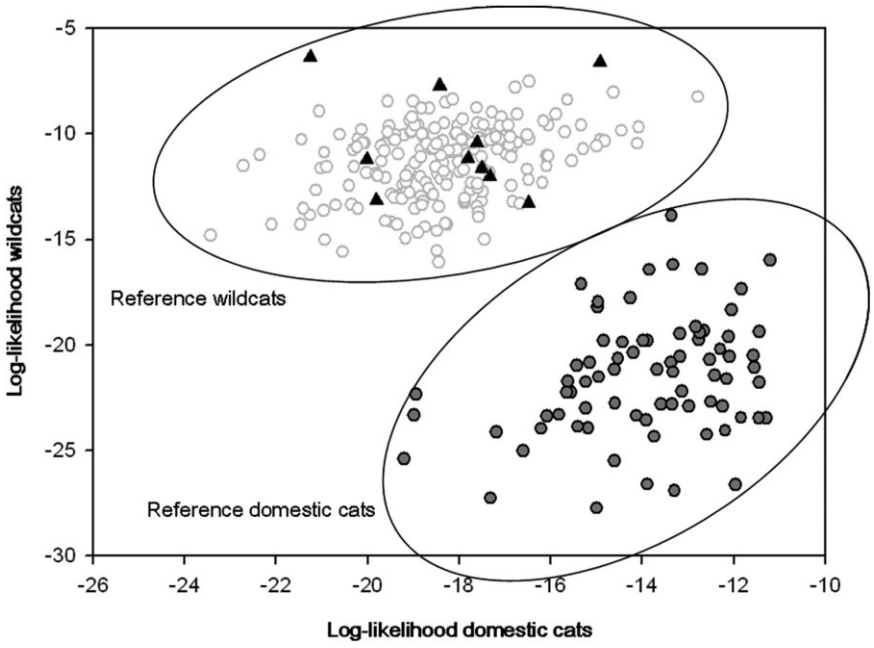

Figure 4 Graphical plotting of individual baseline wildcat (light gray dots), domestic (dark gray dots), and scat (black triangles) microsatellite genotypes of the frequency-based population assignment test performed using software GENALEX. The plot aims to detect the origin population of each individual by comparing the likelihood of multilocus genotype in a set of predetermined populations (the least negative log value indicates the most likely population).

resampled dataset from the camera trap pairs were 4.35 and 3.70 detections/100 trap-days, resulting in a REM-based population density estimate of 0.39 ± 0.03 (Fig. 5). Finally, the SECR model applied to the scat collection dataset yielded $D = 1.36 \pm 0.73$, with $g(0) = 0.02 \pm 0.01$ and $\sigma = 417 \pm 1299$ m (Fig. 5).

Discussion

Even though we used a low camera-trap density over a relatively small sampling area, we were able to identify a high proportion of the resident wildcats. This was supported by the scat genetic analysis, which also confirmed the taxonomic

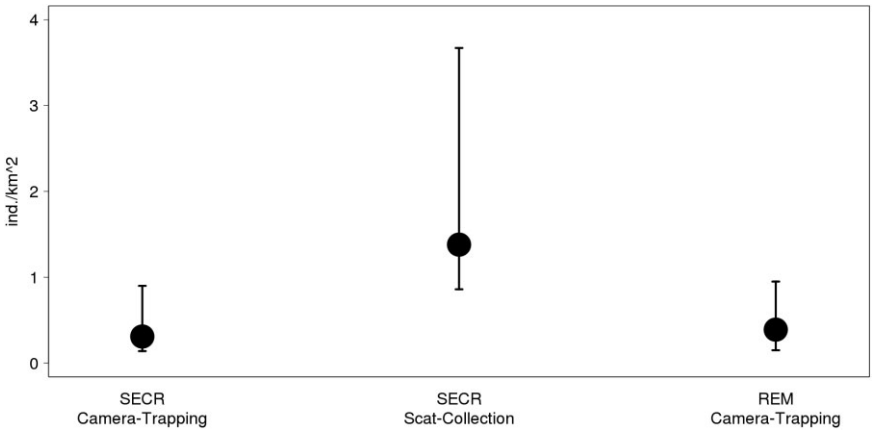

Figure 5 Comparison of density estimates for wildcats in the Etna Regional Park, Italy, obtained by spatially explicit capture-recapture (camera-trapping and scat-collection) and random encounter model (camera-trapping) methods. Whiskers represent the 95% confidence intervals.

status of the cats. While for the camera-trapping dataset, the number of detected individuals versus occasions reached an asymptote, this was not possible for the scat-collection dataset. Indeed, the genetic analysis detected a lower number of individuals and recaptures, which in turn resulted in higher and less precise population density estimates, then those derived from the SECR model and the REM applied to the camera trapping data (Janecka *et al.*, 2011). Nevertheless, the estimated population size derived from the scat dataset for the study area was 14.8 ± 7.9 individuals, which matches the number of individuals identified by camera-trapping. Individual wildcats without recaptures were detected in the outermost camera traps according with the principle that animals with home-ranges inside the trap grid have a higher probability of being photographed (Royle *et al.*, 2009; Kery *et al.*, 2011).

Assuming that fresh scats useful for individual identification have been deposited a maximum of 2 days prior to collection, in principle future studies involving this method could shorten the sampling effort to less than 1 month (e.g. 17 occasions of 2 days). However, we argue that higher sampling effort (transect length and sampling occasions) would actually be required by this method to achieve a more accurate and precise estimation because few recaptures were detected, likely also due to our low DNA extraction success (36%).

The population density estimate from the REM (0.39 ± 0.03 individuals km^{-2}) corresponded closely with that obtained by the SECR analysis of the camera-trapping data (0.32 ± 0.1 individuals km^{-2}). Nonetheless, this finding needs to be considered with caution (Sollmann *et al.*, 2013*b*) as the REM remains relatively little tested under field conditions (Rovero & Marshall, 2009). Furthermore, our SECR and REM estimates cannot be considered completely independent as both relied on data obtained from the same cameras.

The trial calibration of the REM is an essential step (Rowcliffe *et al.*, 2011; Manzo *et al.*, 2012) to derive reliable detection parameters for the camera. Interestingly, the rate of success in triggering the camera by the domestic cat (1/3) approximates that recorded for the wildcat by the pairs of camera trap in the field (19/70); this suggests that our estimation of the camera detection zone should be considered realistic.

The SECR camera-trapping density estimate of our wildcat population (which, according to the earlier considerations, appeared to be the most reliable method) falls within the medium-high values reported in the literature (0.1–0.5 individuals km^{-2}) (Anile *et al.*, 2012), although differences among studies in methodology and genetic identity (hybrid vs. wildcat) of the target population should be taken into account. Similar camera-trapping studies on wildcats have been conducted in Scotland on a hybridizing population (Kilshaw & Macdonald, 2011) and in Turkey (Can *et al.*, 2009). Overall, these studies resulted in lower trapping rates (# detections/100 trap-days; 0.8 and 2.3 in Scotland, and 1.8 in Turkey), lower densities (0.29 and 0.22 individuals km^{-2}) and lower number of individuals identified (13 and 8, respectively). Moreover, their population density estimates were also less precise (SE = 0.13 and 2.9 individuals km^{-2}, respectively) than the one obtained by our SECR camera-trapping method.

Thus far, the only study on wildcats that has used a scat-collection followed by genetic analyses (but not accompanied by population density estimation) is by Piñeiro & Barja (2012) in Spain. These authors report that 16 wildcats were identified from 26 out of 41 scats, yielding a DNA extraction success of 63.4%, which may have been favored by the lower average temperatures.

Our genetic analysis identified 10 European wildcat individuals using nine STRs molecular markers from 14 out of 39 scat samples (36%) collected in this study (Supporting Information Appendix S1). Although we used the multitube approach and strict quality controls required to control for PCR errors associated with low-content DNA samples such as feces, genotyping success in our study was lower (36 % vs. ~70%) than other noninvasive felid DNA studies (Ernest *et al.*, 2000; McKelvey *et al.*, 2006; Janecka *et al.*, 2008; Mondol *et al.*, 2009). As the temporal distribution of the scats positive for the DNA extraction was highly skewed, it is possible that the lower ambient temperatures of May and September (~20°C) versus those in June–August (~30°C) could have reduced the

DNA degradation process, thus resulting in a higher likelihood of DNA extraction.

Despite the short microsatellites motif repeat (all loci were dinucleotides), the significant variability of the loci used in this study, expressed as gene diversity He, (see Randi *et al*., 2001; Pierpaoli *et al*., 2003; Mattucci *et al*., 2013), may have increased the risk of ADO independent of amplicon length and repeat motif [a 10% increase in He would increase ADO rate from 20% to 22.2%, (Broquet *et al*., 2007)]. Improving noninvasive collection protocols (Ruell & Crooks, 2007) and PCR methods will likely increase amplification success and reduce errors, thus making more efficient use of low-quality DNA samples (Janecka *et al*., 2008). In particular, to improve the genotyping success, it could be useful to (1) collecting excremental samples on a daily base to ensure their freshness (Piggott & Taylor, 2003); (2) conducting the survey in period with low ambient temperatures (Piggott & Taylor, 2003); (3) performing the laboratory analyses as soon as possible (Waits & Paetkau, 2005).

Conclusions

Despite the sampling limitations of this study, we could reliably compare population density estimations and hence provide a useful example for designing similar studies, bearing in mind that surveying a larger area and including habitat covariates would strengthen the outcome. While the most reliable method to estimate wildcat population density was the SECR analysis applied to the camera-trapping data, our genetic analysis confirmed the genetic status of our target population. This is important as hybridization with domestic cats is one of the major threats to wildcat conservation (Oliveira *et al*., 2008). The difference in population density estimates from the camera-trapping and scat-collection methods is likely due to the lower number of captures and recaptures obtained from the latter method; this suggests that increasing both sampling effort and genotyping success could increase method performance. Camera-trapping (along with the wide variety of analytical methods recently proposed) can be integrated with genetic tools for noninvasive samples to provide a deeper view into the ecology of the target species (Sollmann *et al*., 2013*a*); such a unified tool can be applied to survey also other mesocarnivore species. Finally, we hope that our findings will contribute to the long-lasting conservation of this threatened and highly distinct insular population of wildcats.

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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:

Appendix S1. Details of the results for the genetic analysis.