



Research Article

A non-invasive monitoring on European wildcat (*Felis silvestris silvestris* Schreber, 1777) in Sicily using hair trapping and camera trapping: does scented lure work?

Stefano ANILE^{a,*}, Carmelo ARRABITO^a, Maria Vittoria MAZZAMUTO^a, Davide SCORNAVACCA^a, Bernardino RAGNI^b

^aDipartimento di Biologia, Università di Catania, via A. Longo 19, 95125 Catania, Italy

^bDipartimento di Biologia Cellulare ed Ambientale, Università degli Studi di Perugia, via Elce di Sotto, 06123 Perugia, Italy

Keywords:

hair trapping
camera trapping
valerian tincture
Etna
wildcat

Article history:

Received: 30 May 2011

Accepted: 27 July 2012

Acknowledgements

Authors thanks: Carlo Amico (Ripartizione Faunistico Venatoria di Catania) that allowed to use camera traps; his family for support and E. Centofanti for camera trap assistance. We thank two anonymous reviewers and the associated editor Damiano Preatoni for their valuable advices that improve the first version of the manuscript.

Abstract

An hair trapping protocol, with camera trapping surveillance, was carried out on the south-western side of the Etna, inhabited by an abundant population of the European wildcat. We aimed to collect hair for genetic analysis on the base of a field study conducted in Switzerland, where valerian tincture had been used to attract wildcats to rub against wooden sticks and therefore leaving hairs. We placed 18 hair trapping stations, plus one camera trap per scented wooden stick, 1 km away from each other for 60 days (October 29 2010 to December 28 2010). The rate of “capture” success (1 capture / 24.5 trap-days) by camera trapping was substantially the same as those obtained during previous surveys performed in the same study area without the use of any attractants. No wildcats were photographed while rubbing against the wooden sticks, neither any wildcat was interested in the scent lure. We discuss limitations of the hair trapping, providing possible explanations on the failure of valerian tincture, while suggesting some field advices for future monitorings.

Introduction

Field study methods are often difficult to implement for species that are rare, endangered and/or cryptic. Using invasive study methods such as trapping, often accompanied by tissue or blood samples collection, might be impossible (Bizzarri et al., 2010). For these reasons assessing the presence/absence or the status of such species can be really difficult. In spite of this, collection of samples by means of non-invasive techniques, such as hair, faeces or camera trapping, can be useful to obtain information about the status or the abundance of a rare and sensitive species. The

use of non-invasively collected samples as sources of DNA for molecular genetic marker analysis can unequivocally determine the species. The possibility to identify individuals and their gender improves the accuracy of abundance estimates, also allowing home-range size, habitat, dietary preferences and social interaction estimates (Piggott and Taylor, 2003).

Recently there has been keen interest in the non-invasive collection of genetic samples, as an alternative to blood or tissue samples taken from trapped animals, because these techniques are generally less expensive and provide data without coming into direct contact with the animal (Barea-Azcon et al., 2007). Hair samples (Valderrama et al., 1999) have been collected from American black bear (*Ursus americanus*) (Woods et al., 1999), pine marten (*Martes martes*) (Lynch et al., 2006), American mink (*Neovision vison*) and European polecat (*Mustela putorius*) (Gonzalez-Esteban et al., 2006). In the field, scents ap-

*Corresponding author

Email addresses: stefanoanile@yahoo.com (Stefano ANILE), carmelo.arrabito@hotmail.it (Carmelo ARRABITO), mariavmazzamuto@gmail.com (Maria Vittoria MAZZAMUTO), davidescornavacca@gmail.com (Davide SCORNAVACCA), lynx@unipg.it (Bernardino RAGNI)

plied to hair traps have assisted biologists in attracting felids to collect hair samples from Canada lynx (*Lynx canadensis*) (Turbak, 1998), ocelots (*Leopardus pardalis*) (Weaver et al., 2003), cheetahs (*Acinonyx jubatus*), leopards (*Panthera pardus*) and lions (*Panthera leo*) (Thomas et al., 2005) and from jaguarondi (*Puma yagouaroundi*) and margay (*Leopardus wiedii*) (García-Alaníz et al., 2010).

In Switzerland Kery and colleagues (2011) conducted an extensive and successful research on the European wildcat (*Felis silvestris silvestris*) based principally on hair-trapping methods using valerian tincture (*Valeriana officinalis*) as attractant. In another study, conducted in a typical Mediterranean landscape (Granada, Spain), a comparison was made among four broadly applied methods for surveying carnivores: in the case of wildcats, both sign surveys and scent stations had low efficiency for detecting the species, whilst a relatively higher efficiency was obtained both for camera-trapping and box-trapping (Barea-Azcon et al., 2007).

Valerian tincture was even used in a field test performed in Scotland, where camera traps have been used to check whether wild living cats were attracted (Kilshaw and Macdonald, 2011).

Valerian tincture has also been experimented in captivity on 9 individuals of Italian *F. s. silvestris* from Central Apennines (n = 6), Sicily (n = 2) and Sardinia (n = 1); in 84.4% of the 135 observations the cats showed a very high degree of excited interest in the lure, throughout the four seasons (Ragni, unpublished observations).

Camera trapping became an efficient and widespread method to assess abundance of a large variety of species, aiming at estimate density of felids like tigers (*Panthera tigris*, Karanth 1995; Jackson et al. 2005), ocelot (*Leo-*

pardus pardalis, Trolle and Kery 2003), jaguars (*Panthera onca*, Silver et al. 2004; Harmsen et al. 2009) and leopards (*Panthera pardus*, Wand and Macdonald 2009). This method is based upon established procedures for capture and recapture analysis of a closed population (Karanth, 1995; Karanth and Nichols, 1998; Karanth et al., 2004) using cameras in place of traps and using the natural coat colour and markings system (Ragni and Possenti, 1996) to recognize pictures of different individuals or to identify “recaptures”, when applied to wildcat. The application of such camera-based capture-recapture techniques in our study area yielded the following density estimates (wildcat/100 ha \pm S.E): 0.45 (2006), 0.46 \pm 0.13 (2007), 0.28 \pm 0.1 (2009) and 0.31 \pm 0.1 (2010) (Anile et al., 2009, 2010, 2012a,b).

Sicily is characterized by the presence of the only insular Mediterranean population of the threatened European wildcat (Ragni, 2006) which is not the result of human introduction (Kitchener and Rees, 2009). Deforestation, habitat fragmentation, illegal hunting and hybridization with the domestic cat (*Felis silvestris catus*) are the major threats to the long term survival of this feline (Nowell and Jackson, 1996). Thus, evaluating wildcats presence and abundance is essential for their conservation and management.

The aim of this study, performed in a typical Mediterranean area, was to collect hairs from wildcats that, attracted by valerian tincture, would have rubbed against wooden sticks leaving hairs for genetic analysis.

Materials and Methods

The study area is located on the south-western side of Mount Etna (north-eastern Sicily), at altitudes ranging from 900 to 2000 m a.s.l. (Fig. 1)

The landscape consists of quite recent large lava flows and volcanic cones of different ages intermixed with wide patches of forest (*Pinus laricio*, *Quercus pubescens* and *Q. ilex*). Human activity is limited to forest management and sheep husbandry. The climate is typically Mediterranean but in winter snow cover can be thick and persistent.

The placement of camera traps and hair traps was determined on the basis of previous studies (Anile et al., 2009, 2010, 2012a). Eighteen digital camera traps (DFV®7.2 megapixels) with passive infrared motion/heat sensors were used to monitor 18 different stations, 1 km apart from each other, as to cover an area of 1090 ha (Minimum Convex Polygon) (Fig. 1). Each camera was put 40-60 cm above the ground, in an iron box closed with a padlock and tied to a tree with a chain to avoid damage and theft: cameras were set on 10 s delay between successive photos.

Hair traps consisted of wooden stakes 40 cm long attached to the base of tree trunks. Each wooden stake was carved to snatch and retain hair samples. A cork, submerged for 1 week in valerian tincture, was stuck in a hole in the upper part of the stake (Fig. 2).

The hair traps were watched by camera traps in order to assess the passage of wildcats and the possible rubbing against the

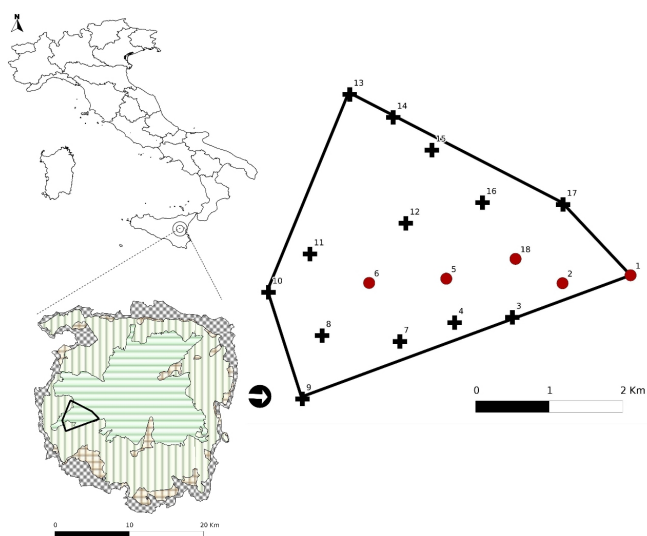


Figure 1 – Location of Sicily (top) and boundaries of the protection zones within the Etna Regional Park (bottom). In detail: camera traps positive (crosses) and negative (points) for wildcat; polygon area (MCP) = 1080 ha. Arrow indicates the station where domestic cat was photographed.



Figure 2 – A wooden stick used in this study.

sticks. The monitoring was carried out from 29 October 2010 to 28 December 2010 (60 days); cameras control and change of corks were done once a week.

Results and discussion

Camera traps and hair traps worked for a total of 1080 trap-days. Camera trapping recorded 44 wildcat events for 13 out of 18 stations, and 2 events of a free ranging domestic cat were detected by one station located in the south-western corner of the study area (Fig. 3, Tab. 1).

Table 1 – Number of positive events per station. Easting, Northing: UTM coordinates (zone 32, datum WGS84); starting, ending: monitoring period; N: number of pictures obtained from each station.

Station	Easting	Northing	Starting	Ending	N
1	493127	4176420	29/10/2010	28/12/2010	
2	492201	4176312	29/10/2010	28/12/2010	
3	491505	4175875	29/10/2010	28/12/2010	6
4	490719	4175804	29/10/2010	28/12/2010	5
5	490619	4176374	29/10/2010	28/12/2010	
6	489569	4176316	29/10/2010	28/12/2010	
7	489974	4175547	29/10/2010	28/12/2010	1
8	488916	4175628	29/10/2010	28/12/2010	4
9 ¹	488647	4174766	29/10/2010	28/12/2010	2
10	488185	4176216	29/10/2010	28/12/2010	3
11	488750	4176737	29/10/2010	28/12/2010	3
12	490055	4177155	29/10/2010	28/12/2010	4
13	489291	4178907	29/10/2010	28/12/2010	2
14	489883	4178595	29/10/2010	28/12/2010	1
15	490410	4178149	29/10/2010	28/12/2010	11
16	491098	4177436	29/10/2010	28/12/2010	1
17	492195	4177410	29/10/2010	28/12/2010	1
18	491561	4176643	29/10/2010	28/12/2010	

¹ station that photographed domestic cat

The overall photo-capture success rate, excluding the domestic cat, was 1 capture/24.5 trap-days, but we didn't collect any cat hair samples during the whole study because wildcats didn't rub against the sticks, neither showed any kind of interest in the scent lure.

Even other carnivore wildlife species present in the area (e.g. pine marten *Martes martes* and red fox *Vulpes vulpes*), did not show any kind of reaction due to the presence of the scent lure.

We can affirm, analyzing the data set of wildcat pictures obtained, that no animal seemed to be interested in valerian tincture scent. In their study Kery et al. 2011 assessed that valerian tincture is most efficient from November to April with maximum success in March, so our monitoring period (November-December), being out of wildcat mating period (from December to March), might have negatively influenced the likelihood to collect hair samples. Furthermore, hair trapping failure was not probably due to the use of the camera flash during the night: in the cited Swiss study some of the sticks were monitored by camera-trapping equipped with a flash and several "flashed" pictures of wildcat rubbing against sticks were gained (Raydelet, 2009). Moreover, two events were obtained in the morning and wildcats didn't seem to be interested in the lure as well. Additionally, one wildcat passed (again showing no interest for the scent) in front of an hair trap just one hour after the cork replacement (Fig. 3). Finally, the long term camera-trapping project conducted in the same study area did not highlight the occurrence of trap-avoidance behavior (Anile et al., 2009, 2010, 2012a,b) because several recaptures were obtained during each monitoring.

Interestingly in a similar test carried out in Scotland (Kilshaw and Macdonald, 2011), the use of valerian tincture did not produce any kind of result, both in term of hair trapping and camera-trapping success: no pictures were obtained during the field test with this kind of lure.

Our study proves that while camera trapping efficiency for monitoring wildcats is reasserted, hair trapping is not reliable even using scent lures: in our case valerian tincture, used as a lure to encourage wildcats to rub, did not work: in 44 wildcat detections events we did not see any sort of interest in this kind of bait.

In addition to the possible explanation of failure above, due to the monitoring period, we propose some other possible biological explanations: we argue that failure could be due also to climatic and ecological characteristics (continental climate in Switzerland versus Mediterranean climate in Sicily); furthermore, the attractiveness of valerian tincture such as liquid catnip is genetically related: individuals may or may not be predisposed to be attracted (about 2/3 of cats are susceptible to catnip, Waller at al. 1969). Considering that Sicily is an island, it's likely that the Etna's wildcat population could not be genetically predisposed to be attracted by valerian tincture: this could be



Figure 3 – Six passages of wildcat detected by camera trapping during the day and the night. Red circles highlight the wooden stick.

due to a bottleneck effect (Mattucci et al., *in preparation*) caused by the drastic decrease of the wildcat population before the species was protected and Mt. Etna Natural Park was established.

One of the major flaws of hair trapping field protocols is obtaining samples with enough DNA (Piggott and Taylor, 2003) for analysis, despite the relative abundance of DNA in feces, especially on the surface of those still fresh: for example feces from mountain lions (*Puma concolor*) were used to collect genetic information where the DNA comes from a few sloughed intestinal cells (Ernest, 2000).

We believe that feces collection could represent an alternative and complementary (providing information on the diet) method to hair trapping as source of DNA for molecular genetic marker analysis, such as for the Iberian lynx (*Lynx pardinus*, Paolmares et al. 2002) and for other small-sized carnivores in Italy (for examples see Balestrieri et al. 2008; Lucentini et al. 2007; Vercillo et al. 2005).

We also want to highlight the first documented presence of a domestic cat into our study area during five years of camera trapping: this indicates a crucial problem for the future long-term conservation of wildcat in Sicily and camera-trapping could be a valuable method to detect domestic cat presence inside areas typically inhabited by wildcats (Sarmiento et al., 2009).

In conclusion, based on the results of this pilot study, we strongly recommend, before starting a massive wildcat hair trapping monitoring program, to conduct a trial test using camera trapping to verify the effectiveness of any attractants. Furthermore, hair-trapping appears to be a non-invasive method whose success is highly correlated to species, period of monitoring and ecological characteristics of the study area. Hair trapping protocols have to

be strictly focused on the target population, while camera trapping can detect many kinds of species in different habitats under any kind of weather conditions (wind, rain, snow etc.).

References

- Anile S., Bizzarri L., Ragni B., 2009. Camera trapping the European wildcat (*Felis silvestris silvestris*) in Sicily (Southern Italy): preliminary results. *Hystrix It. J. Mammal.* 20(1): 55–60.
- Anile S., Bizzarri L., Ragni B., 2010. Estimation of European wildcat population size in Sicily (Italy) using camera-trapping and capture-recapture analyses. *Ital. J. Zool.* 77(2): 241–246.
- Anile S., Amico C., Ragni B., 2012a. Population density estimation of the European wildcat (*Felis silvestris silvestris*) in Sicily using camera trapping. *Wildl. Biol. Pract.* 1(8): 1–11. doi:10.2461-wbp.2012.8.1
- Anile S., Ragni B., Randi E., Mattucci F., 2012b. Spatial explicit capture recapture model apply to camera trapping and genetic scat survey on the wildcat from Sicily. *Proceedings of the VIII Italian Congress of Teriology 9–11 May 2012 Piacenza (PC)*.
- Balestrieri A., Ruiz-Gonzalez A., Remonti L., Gomez-Moliner B.J., Genovese S., Gola L., Prigioni C., 2008. A non-invasive genetic survey of the pine marten (*Martes martes*) in the Western River Po Plain (Italy): preliminary results. *Hystrix It. J. Mammal.* 19(1): 77–80.
- Barea-Azcon J., Virgos E., Ballestreros-Duperon E., Moleon M., Chiroso M., 2007. Surveying carnivores at large spatial scales: a comparison of four broad-applied methods. *Biodivers. Conserv.* 16: 1213–1230.
- Bizzarri L., Lacrimini M., Ragni B., 2010. Live capture and handling of the European wildcat in central Italy. *Hystrix It. J. Mammal.* 21(1): 73–82.
- Ernest H.B., 2000. DNA sampling and research techniques. *Outdoor California* 61(3): 20–21.
- García-Alaníz N., Naranjo E.J., Mallory F.F., 2010. Hair-snares: A non-invasive method for monitoring felid populations in the Selva Lacandona, Mexico. *Trop. Conserv. Sci.* 3(4): 403–411.

- Gonzalez-Esteban G., Villate I., Irizar I., 2006. Differentiating hair samples of the European mink (*Mustela lutreola*), the American mink (*Mustela vison*) and the European polecat (*Mustela putorius*) using light microscopy. *J. Zool.* 270: 458–461.
- Harmsen B.J., Foster R.J., Silver S.C., Ostro L.E.T., Doncaster C.P., 2009. Spatial and temporal interactions of sympatric jaguars (*Panthera onca*) and pumas (*Puma concolor*) in a neotropical forest. *J. Mammal.* 90(3): 612–620.
- Jackson R.M., Roe J.D., Wangchuk R., Hunter D.O., 2005. Surveying snow leopard populations with emphasis on camera trapping: a handbook. 73.
- Karanth K.U., 1995. Estimating tiger populations from camera-trap data using capture-recapture models. *Biological Conservation* 71: 333–338.
- Karanth K.U., Nichols J.D., 1998. Estimation of tiger densities in India using photographic captures and recaptures. *Ecology* 79(8): 2852–2862.
- Karanth K.U., Chundawat R.S., Nichols J.D., Samba Kumar N.S., 2004. Estimation of tiger densities in the tropical dry forests of Panna, Central India, using photographic capture-recapture sampling. *Anim. Conserv.* 7: 285–290.
- Kery M., Gardner B., Stoeckle T., Weber D., Royle J.A., 2011. Use of spatial capture-recapture modelling and DNA data to estimate densities of elusive animals. *Conserv. Biol.* 25(2): 356–364.
- Kilshaw K., Macdonald D.W., 2011. The use of camera trapping as a method to survey for the Scottish wildcat. Scottish Natural Heritage Commissioned Report n° 479.
- Kitchener A.C., Rees E.E., (2009). Modelling the dynamic biogeography of the wildcat: implications for taxonomy and conservation. *J. Zool.* 279: 144–155.
- Lucentini L., Vercillo F., Palomba A., Panara F., Ragni B., 2007. A PCR-RFLP method on faecal samples to distinguish *Martes martes*, *Martes foina*, *Mustela putorius* and *Vulpes vulpes*. *Conserv. Genet.* 8: 757–759.
- Lynch A.B., Brown M.J., Rochford J.M., 2006. Fur snagging as a method of evaluating the presence and abundance of a small carnivore, the pine marten (*Martes martes*). *J. Zool.* 270: 330–339.
- Nowell K., Jackson P., 1996. Wild Cats: Status survey and conservation action plan. International Union for Nature Conservation (IUCN) / Cat Specialist Group, Gland, Switzerland. pp. 110–113. The Burlington Press, Cambridge, UK.
- Palomares F., Godoy J.A., Piriz A., O'Brien S.J., Johnson W.E., 2002. Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. *Mol. Ecol.* 11: 2171–2182.
- Piggott M.P., Taylor A.C., 2003. Remote collection of animal DNA and its application in conservation management and understanding the population biology of rare and cryptic species. *Wildl. Res.* 30: 1–13.
- Ragni B., Possenti M., 1996. Variability of coat-colour and markings system in *Felis silvestris*. *Ital. J. Zool.* 63: 285–292.
- Ragni B., 2006. Il gatto selvatico. In: Salvati dall'arca. WWF Italia, Edizioni A. Perdisa, Bologna pp 35–56. [in Italian]
- Raydelet P., 2009. Le chat forestier. Delachaux et Niestlé, Paris. [in French]
- Sarmiento P., Cruz J., Eira C., Fonseca C., 2009. Spatial colonization by feral domestic cats *Felis catus* of former wildcat *Felis silvestris silvestris* home ranges. *Acta Theriol.* 54(1): 31–38.
- Silver S.C., Ostro L.E.T., Marsh L.K., Maffei L., Noss A.J., Kelly M.J., Wallace R.B., Gomez H., Ayala G., 2004. The use of camera traps for estimating jaguar *Panthera onca* abundance and density using capture/recapture analysis. *Oryx* 38(2): 148–154.
- Thomas P., Balme G., Hunter L., McCabe-Parodi J., 2005. Using scent attractants to non-invasively collect hair samples from cheetahs, leopards and lions. *Anim. Keeper's Forum* 7/8: 342–384.
- Trolle M., Kery M., 2003. Estimation of ocelot density in the Pantanal using capture-recapture analysis of camera-trapping data. *J. Mammal.* 84(2): 607–614.
- Turbak, G. 1998. Seeking the missing lynx. *National Wildlife.* 36: 19–24.
- Valderrama X., Karesh W.B., Wildman D.E., Melnick D.J., 1999. Noninvasive methods for collecting fresh hair tissue. *Mol. Ecol.* 8: 1749–1752.
- Vercillo F., Lucentini L., Mucci N., Ragni B., Randi E., Panara F., 2005. A simple and rapid PCR-RFLP method to distinguishing *Martes martes* and *Martes foina*. *Conserv. Genet.* 5: 869–871.
- Waller G.R., Price G.H., Mitchell E.D., 1969. Feline attractant, cis,trans-nepetalactone: metabolism in the domestic cat. *Science* 164(3885): 1281–1282.
- Wang S.W., Macdonald D.W., 2009. The use of camera traps for estimating tiger and leopard populations in the high altitude mountains of Bhutan. *Biol. Conserv.* 142: 606–613.
- Weaver J., Wood P., Paetkau D., 2003. A new non-invasive technique to survey ocelots. *Wildl. Conserv. Soc. Publ.* 22 pp.
- Woods J.G., Paetkau D., Lewis D., McLellan B.N., Proctor M., Strobeck C., 1999. Genetic tagging of free-ranging black and brown bear. *Wildl. Soc. B.* 27: 616–627.

Associate Editors: D.G. Preatoni